

USING FUNCTIONAL TRAITS TO UNDERSTAND HOW CHANGES IN PLANT
SPECIES COMPOSITION WILL AFFECT LARVAL AMPHIBIANS

A Dissertation

Presented to the Faculty of the Graduate School
of Cornell University

In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by

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May 2013

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Cornell University 2013

The vast majority of plant material becomes detritus, and in its ‘afterlife’ this plant litter is a critical resource for many aquatic organisms. I conducted four experiments to improve our understanding of how changes in plant communities may affect aquatic organisms. In each experiment, I used larval anurans as a focal organism, since many studies show that larval anurans are sensitive to changes in litter inputs. All experiments were conducted in outdoor mesocosms containing plant litter, microbes, algae, and tadpoles. In all cases, I predicted that as litter quality or quantity increased, tadpole performance – measured as survivorship, development rate, and body size - would improve. In one experiment, I varied litter quantity and observed that tadpole development and size increased with litter quantity. In a second experiment, I examined how the presence of a predator affected tadpole performance. I predicted that predators would decrease tadpole performance, but that these negative effects would attenuate as litter quality increased. I found that tadpole survival and mass increased with litter quality but saw no apparent effects of predators. In a third experiment I collected soil from six different sites and created inocula for outdoor mesocosms to see whether tadpole performance would vary among inocula. Tadpoles developed faster and achieved greater body size when raised with higher quality litter, but I did not detect differences in tadpole performance among soil inocula. In a fourth experiment, I used litter from six plant species to create nine unique mixtures of three

species each. I predicted that as plant functional diversity increased, tadpole performance would improve. I also predicted that tadpole performance would improve as community-weighted mean (CWM) nitrogen and phosphorus increased and CWM lignin and phenolics decreased. I did not observe any differences in tadpole performance based on functional diversity, but found that tadpole performance improved as CWM nitrogen increased and CWM phenolics decreased. My work shows that plant litter quality is a consistent predictor of larval anuran performance, even when there are multiple plant species, predators, or variation in soil biota communities.

BIOGRAPHICAL SKETCH

Jillian Standish Cohen decided to become an environmental scientist at the age of 9, when a man with a banjo came to her elementary school and sang songs about the animals in the Chesapeake Bay. Earning her doctorate in Natural Resources is an important milestone in fulfilling this dream. Jill lives in Alexandria, VA with her husband and two overfed cats.

I would like to dedicate this dissertation to my mother, Marilla Standish Cohen, who taught me to strive to be smart, independent, and considerate of others. And to my father, Lenard Cohen, for making me feel loved every moment of my life.

ACKNOWLEDGMENTS

I would like to thank Dr. Bernd Blossey for his unwavering support and patience, and for letting me forge my own path. I am grateful to Shorna Broussard Allred, Eric Nelson, and Joseph Yavitt for being so generous with their time, resources, and valuable advice. I owe a great debt to my labmates, including Andrea Davalos, Jeremy Dietrich, Annise Dobson, Brian Greene, and Laura Martin, for being a constant source of support. I thank Stacy Biddlecomb, Angel Cassanova-Torres, Inga Conte-Jerpe, Stacey Ng, Shauna-Kay Rainford, Wade Simmons, Charlotte Thurston, and Matt Valente; they helped me directly by assisting me with my research, and indirectly by inspiring me with their intelligence and work ethic. I must also thank Victoria Nuzzo, whose patience, intelligence, cheer, and diligence was always a welcome refuge. I thank Alexander Flecker, Clifford Kraft, and Jim Tantillo for making it a genuine pleasure to TA. My graduate research was funded by generous grants from the National Science Foundation Graduate Research Fellowship (Grant No. DGE-0707428), the New York State Department of Transportation, the Society of Wetland Scientists, the Kieckhefer Adirondack Fellowship, and the Cornell Program in Biogeochemistry and Environmental Biocomplexity. All research conformed to the legal requirements for use of vertebrates in research and was approved by Cornell University's Institutional Animal Use and Care Committee.

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CHAPTER 1

QUANTITY COUNTS: AMOUNT OF LITTER DETERMINES TADPOLE PERFORMANCE IN EXPERIMENTAL MICROCOSMS

Abstract

Terrestrial plant litter is an important subsidy to freshwater ecosystems, where it serves as a basal resource in benthic food webs. Litter quantity and decomposition rate strongly influence energy flow through these food webs. Litter quantity often increases following nonnative plant invasions, but the impact this additional litter has on aquatic consumers is largely unknown. We conducted an experiment in outdoor microcosms containing plant litter, microbes, algae, and tadpoles. Many tadpoles feed on biofilms (bacteria, fungi, and algae) that develop during decomposition; as such, they are ideal organisms to test bottom-up effects of litter subsidies. We used a related pair of native and nonnative wetland grasses to investigate whether litter quantity affects tadpole performance. Tadpole performance metrics (developmental stage, size, and survival) showed significant positive responses to increased litter quantity but were mostly unaffected by plant species. Litter quantity was therefore the primary determinant of tadpole performance in our experimental community. We suggest that changes to litter quantity can have important impacts on larval amphibians.

Introduction

Increased plant diversity is often associated with increased primary productivity (e.g. Tilman et al. 2001, Roscher et al. 2005, Balvanera et al. 2006) and reductions in this diversity are generally assumed to have negative ecological consequences (Zavaleta et al. 2010). Nonnative plant invasions can reduce local diversity, yet contrary to expectations, plant invasions often increase primary productivity (Ehrenfeld 2003, Liao et al. 2007, Liao et al. 2008), giving rise to the so-called “invasion-diversity-

productivity paradox” (Rout and Callaway 2009). This phenomenon is especially apparent in wetlands, where nonnative plant invaders typically establish highly productive monocultures (Zedler and Kercher 2004). For example, Farnsworth and Myerson (2003) examined two nonnative and two native wetland plant species and found that nonnative plants produced up to eight times more biomass compared to native plants.

Since the majority of plant biomass senesces and enters detritus-based food webs (Cebrian 1999), it follows that plant invasions can increase detritus production. Several authors have observed that nonnative wetland plants produce copious amounts of litter (Farrer and Goldberg 2009, Tuchman et al. 2009, Vaccaro et al. 2009). For example, Vaccaro et al. (2009) observed that nonnative cattail (*Typha angustifolia*) produced 1.4 - 6.5 times more litter compared to native wetland plants. Increased litter inputs could have important consequences for detritus-based food webs, especially in freshwater benthic communities, where plant litter is a dominant source of energy supporting consumer productivity (Mann 1988, Bonner et al. 1997, Wallace et al. 1999, Rubbo and Kiesecker 2004, Cole et al. 2006, Rubbo et al. 2006).

As plant litter decomposes, biofilms (fungi, bacteria, and algae) form on litter surfaces, gaining energy and nutrients from the dead plant material. Both decomposition rate and overall litter quantity govern rates of energy flow in these systems (Cebrian and Lartigue 2004), with numerous aquatic animals grazing on biofilms, including many larval amphibians (Altig et al. 2007). Studies investigating how plant invasions affect aquatic consumers via changes in litter decomposition rate have addressed effects of litter chemistry (Bailey et al. 2001, Graça et al. 2001, Thompson and Townsend 2003, Hladysz et al. 2009), plant species richness (Swan et al. 2008), litter structure (Moline and Poff 2008), and detritivore community dynamics (Dangles et al. 2002, Bastow et al. 2008), while holding litter quantity constant.

However, studies that ignore litter quantity may not accurately estimate faunal responses to invasion. Even if plant species have identical litter quality (e.g. carbon: nitrogen: phosphorus ratios, lignin and tannin content), differences in their primary productivity are still critically important for consumer productivity in aquatic ecosystems (Wallace et al. 1997, 1999, Tiegs et al. 2008). Very few studies have explicitly examined the effects of litter quantity on larval amphibians; however, Rubbo et al. (2008) found that increasing leaf litter quantity from native tree species was positively correlated with performance of larval *Lithobates (Rana) sylvaticus* (wood frog), but had no effect on larval salamanders (*Ambystoma* spp.). Given that native and nonnative plants do tend to differ in biomass production, considering how increases in litter quantity may affect detritus-based food webs is critical to understanding how nonnative plant invasions may impact aquatic ecosystems.

We examined the potential bottom-up effects of changes in litter quantity on a tadpole species that is common to the Northeastern USA. We assembled experimental outdoor microcosms using a pair of confamilial native and nonnative grasses. We designed our experiment using species similar in litter quality (nitrogen, phosphorus, and lignin content) and structure allowing us to focus on effects of litter quantity. We used a microcosm design that minimizes environmental differences (e.g. temperature) and excludes other stressors (e.g. interspecific competition, predators). We predicted that as litter quantity increased, tadpole performance would improve.

Materials and Methods

We used litter from either native *Calamagrostis canadensis* (bluejoint) or nonnative *Phalaris arundinacea* (reed canarygrass). These two closely related Poaceae have similar phenologies, growth habits, and structural characteristics (USDA 2002, Darris 2008). While the origin of North American populations of *P. arundinacea* is

somewhat ambiguous, it is widely accepted as nonnative (Merigliano and Lesica 1998, Lavergne and Molofsky 2007). Both species are common constituents of freshwater ecosystems in the Northeastern US (USDA 2002, Darris 2008). In November 2007, we collected senesced litter of both species at Lakeview Wildlife Management Area (Jefferson County, New York, USA) by clipping emergent material (leaves and stems) from several monospecific patches. Though plant material had senesced, at this time it was still free-standing – collecting it in this state ensured that minimal decomposition occurred in the field. We kept litter in separate mesh bags and air-dried the material in a greenhouse under ambient temperature until May 2008 (15-30 °C). We then prepared six random samples of 3 g of ground material (< 1 mm) from each species. We sent three of these to Dairy One in Ithaca, New York, USA (<http://www.dairyone.com>) for nitrogen, phosphorus, and lignin analysis.

To obtain estimates of the range of litter quantity produced by our two study species we collected senesced material at field sites (seven for *P. arundinacea*, three for *C. canadensis*) in central New York, USA. At each site, we removed all above ground biomass in three 30 x 30 cm quadrats, and the weighed air-dried material after two weeks. We calculated productivity/m² (*P. arundinaceae*: range 215.3 – 1096.4 g m⁻², mean = 542.3 ± 55.44 g m⁻²; *C. canadensis*: range 297 - 414 g m⁻², mean = 363 ± 34.6 g m⁻²). Scaling these field biomass values to the surface area of our microcosms (0.031 m²) *P. arundinaceae* ranged from 6.7 - 34.4 g and *C. canadensis* 9.2 - 12.8 g per 0.031 m². Based on these values we created a range of litter quantities spanning the spectrum from low to high: 2, 4, 8, 16, and 32 g for both plant species. We replicated each litter treatment ten times, for a total of 100 microcosms.

Lithobates (Rana) palustris (pickerel frog) is a common North American anuran that lays eggs in small, permanent water bodies such as meadow ponds and streams (Wright 1914, Pough and Kamel 1984). As with many larval anurans,

relatively little is known about *L. palustris*' feeding habits, but plant litter and associated biofilms (bacteria, fungi and algae) likely comprise a significant portion of tadpole diets (Altig et al. 2007). *Lithobates palustris* has a relatively long larval period and a large size at metamorphosis (Pough and Kamel 1984); in some cases larvae may overwinter (Wilbur and Fauth 1990), though in our study region most larvae metamorphose in August and September (Wright 1914, Pough and Kamel 1984). On 9 May 2008, we collected five *L. palustris* egg masses (approx. 500-1,000 eggs per mass) from a pond at the Arnot Teaching and Research Forest in Van Etten, New York, USA. We maintained individual egg masses in 10 L, mesh-covered plastic containers that were floating in a small, shaded, outdoor pool. Eggs began to hatch on 18 May 2008. We provided dry fish flakes ad libitum until tadpoles from all clutches reached the free-swimming stage (stage 25, Gosner 1960).

In early May 2008, we established microcosms at the Cornell Resource Ecology and Management (REM) Facility in Ithaca, New York, USA. Each microcosm consisted of a 10 L transparent bag suspended from an open, floating ring (0.03 m² surface area, 0.50 m length). Bags were made of impermeable plastic, so that water within bags did not exchange with water in the surrounding pool. We randomly assigned microcosms to one of four 2 x 2 x 1 m pools. We enclosed each pool in walk-in field cages (Lumite® screening, 20 by 20 mm mesh size, shade 15%, Synthetic Industries, Gainesville, Georgia, USA) to protect microcosms from outside animals (e.g insect predators or amphibian colonists) then filled the pool with tap water to establish a thermal buffer. We filled each microcosm with 10 L of tap water on 15 May 2008 and allowed water to age for 3 days before adding plant litter. We added litter (10-20 cm long pieces of mixed leaves and stems) to microcosms on 18 May 2008. On this same date, we collected 100 L of pond water from a shallow pond containing a well-established *P. arundinacea* stand at the Cornell Experimental Pond

Facility in Ithaca, New York. We filtered the pond water through 80 μm mesh, continuously homogenized the resulting microbial and algal mix, and added 0.2 L of the mixture to each microcosm. We allowed litter to leach and age for nearly two weeks and on 1 June 2008, we added ten tadpoles (two tadpoles each from five different clutches) to each microcosm.

Beginning 29 May 2008, we measured dissolved oxygen (mg/L), pH, and temperature ($^{\circ}\text{C}$) in the middle water column of each microcosm every two weeks using a YSI 556 MPS (YSI Environmental, Yellow Springs, OH). We terminated the experiment after 15 weeks. At this time, we euthanized all tadpoles in a bath of 2% buffered MS-222 (tricaine methane sulfonate) and preserved them in 90% ethanol. We then measured tadpoles' snout-vent length (SVL), a standard metric of tadpole size, and assessed their development rate (Gosner stage, Gosner 1960).

We performed all statistical analyses using R (R Development Core Team 2011). We conducted Student's t-tests to examine differences in litter chemistry (nitrogen, phosphorus, and lignin) between the two litter species. Dissolved oxygen, pH, and temperature are also known to impact tadpole development (Ultsch et al. 1999). Because a previous experiment (Rubbo et al. 2008) found that litter quantity affected dissolved oxygen levels, we used analysis of variance (ANOVA) to test for differences in dissolved oxygen, pH, and temperature among treatments. In each ANOVA, a single environmental variable served as the dependent variable, while litter species, litter quantity, and their interaction were independent variables. We repeated the process to test for differences in dissolved oxygen, pH, or temperature among pools. In case of overall significant ANOVA we used Tukey's HSD to test for differences among treatments.

Larval amphibians that complete metamorphosis earlier and at a larger size generally have higher fitness compared to conspecifics that metamorphose later and at

a smaller size (Smith 1987, Semlitsch et al. 1988, Berven 1990). We used tadpole developmental stage and SVL as indicators of tadpole performance. To assess how treatments affected tadpole performance, we used generalized linear mixed-effect models where litter species and litter quantity were treated as fixed effects and permitted to interact. We treated pool as a random effect. We created three models, with either tadpole count, Gosner stage, or SVL as a dependent variable. We assumed a Poisson distribution for tadpole count data and normal distributions for Gosner stage and SVL. Where appropriate, we verified that assumptions of normality and homoscedasticity were met.

Results

Litter chemistry was similar for both plant species. Litter N and P content did not differ significantly ($t = 0$, $df = 4$, $P \approx 1$) between *P. arundinacea* and *C. canadensis*, with litter containing 1% N and 0.007% P. However, the mean lignin content in *C. canadensis* was 8.17% compared to 4% in *P. arundinacea*, a significant difference ($t = 13.1$, $df = 2$, $P < 0.001$). The mean temperature in microcosms was 21 °C (± 0.01 SE), pH 7.7 (± 0.03 SE), and dissolved oxygen 7.6 mg/L (± 0.16 SE). Dissolved oxygen varied significantly among litter quantity treatments ($F_{1,96} = 117$, $P < 0.001$, Figure 1.1); treatments with 2, 4, and 8 g of litter had significantly higher levels of dissolved oxygen compared to treatments with 16 and 32 g of litter (Tukey's HSD, Figure 1.1) but did not vary among pools (data not shown). There was also a significant litter species by litter quantity interaction ($F_{1,96} = 17.1$, $P < 0.001$, Figure 1.1). Temperature and pH did not vary significantly among treatments (data not shown) but temperature differences among pools were significant although differences were $< 1^\circ\text{C}$ ($F_{3,96} = 63.0$, $P < 0.001$, Figure 1.2A).

At the end of the experiment, 80% of microcosms had at least one surviving tadpole. Across all replicates, survival rate was low - at just over 22%. Overall, litter quantity had a positive effect on tadpole performance. For the Poisson model of tadpole counts, litter species, litter quantity, and their interaction had no effect (Figure 1.3A). Linear mixed-effect models on tadpole Gosner stage and SVL showed that litter quantity had a positive effect on both these metrics of tadpole performance (Table 1.1, Figures 1.3B and 1.3C). Litter species alone did not have an effect on Gosner stage or SVL, but the interaction between species and quantity had a significant effect on tadpole Gosner stage (Table 1.1, Figure 1.3B).

Table 1.1. Linear mixed-effects model results for the effects of litter species and quantity on tadpole performance at 15 weeks.

Response	Litter species			Litter quantity			Litter species x quantity		
	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
Gosner stage	73	-0.88	0.380	73	5.51	<0.001	73	2.64	0.010
Snout-vent length (mm)	73	-0.04	0.965	73	4.99	<0.001	73	0.64	0.524

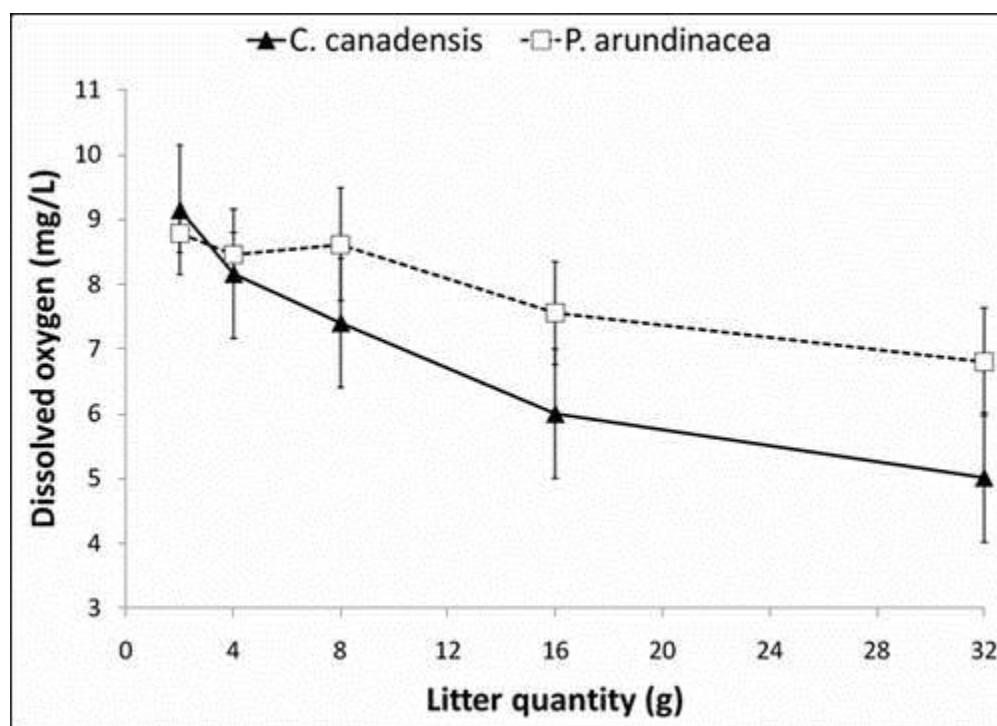


Figure 1.1 Dissolved oxygen (mg/L) in aquatic microcosms along a gradient of litter quantity for *C. canadensis* and *P. arundinacea*. Data are means $\pm 2SE$ of 10 replicates per treatment.

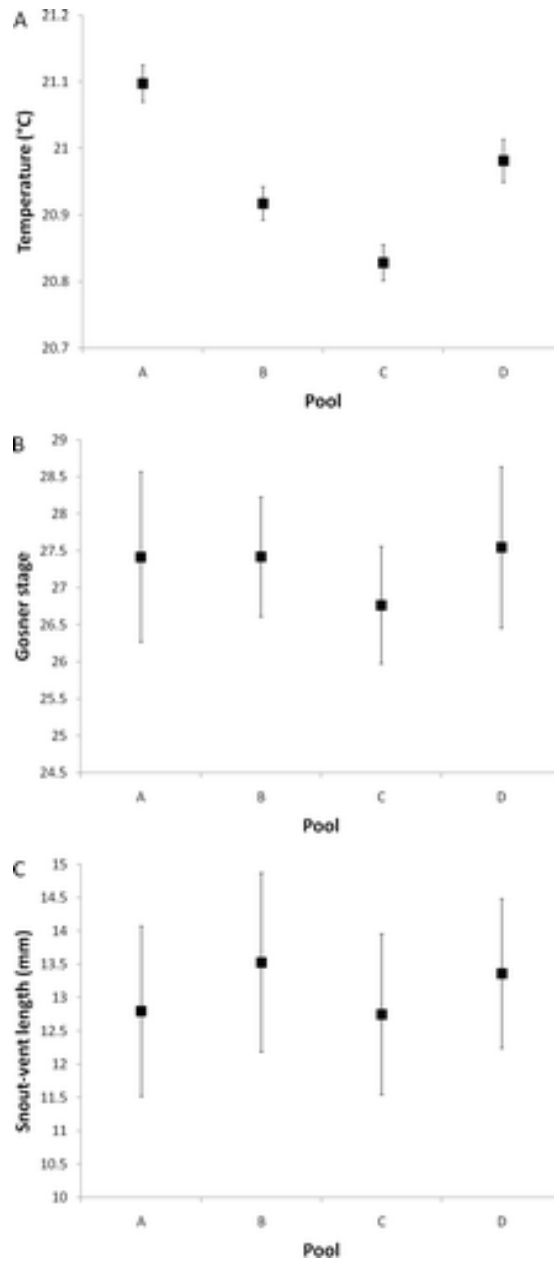


Figure 1.2 Differences in (A) temperature (°C); (B) *Lithobates palustris* Gosner stage at 15 weeks; and (C) *L. palustris* snout–vent length (mm) at 15 weeks among experimental pools. Data are means \pm 2 SE of 10 replicates per treatment.

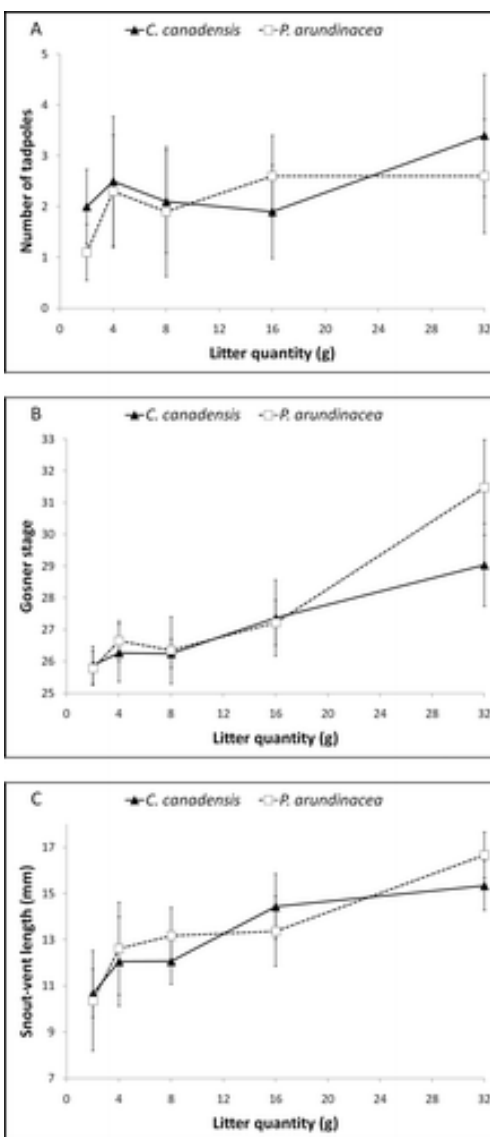


Figure 1.3 Tadpole performance as a function of litter quantity for *Calamagrostis canadensis* and *Phalaris arundinacea*. (A) Number of tadpoles; (B) Gosner stage at 15 weeks; (C) snout–vent length at 15 weeks. Data are means \pm 2 SE of 10 replicates per treatment.

Discussion

We observed significant effects of plant litter quantity across all metrics of tadpole performance, highlighting litter quantity as an important but often overlooked factor in assessing ecological impacts of plant invasions, regardless of plant origin. Litter quantity was the only significant predictor of tadpole performance in several of our analyses, while litter species by itself was never significant.

Our study contributes to a larger body of work demonstrating the importance of plant litter to larval amphibian development (e.g. Maerz et al. 2005, Brown et al. 2006, Rubbo et al. 2008, Williams et al. 2008, Maerz et al. 2010a). Many of these studies find that litter quality is a key determinant of larval amphibian performance. While the qualities of *P. arundinacea* and *C. canadensis* litter used in this study were similar, they were not identical, and differences in litter chemistry likely influenced *L. palustris* performance. For example, tadpoles reared with 32 g of *P. arundinacea* developed faster than tadpoles reared in 32 g of *C. canadensis* (Figure 1.3B). *Phalaris arundinacea* had much lower lignin content compared to *C. canadensis*; it may have decomposed faster and supported a more productive biofilm for tadpoles to feed on. Litter quality can vary considerably within species, enough to alter decomposition rates (Lecerf and Chauvet 2008), so it would be very difficult to control for litter quality completely. Importantly, our goal in this study was not to refute that litter quality influences larval amphibians, but to investigate whether litter quantity may also be important. Future studies could assess the relative importance of effects of litter quantity and litter quality on amphibian performance.

Our results suggest that litter quantity can affect tadpole performance, but the underlying mechanisms are unclear. While we surmised that increased litter quantity would increase food availability to tadpoles (i.e. biofilm production), we did not explicitly measure tadpole feeding rates or food availability. If litter quantity does

indeed influence food availability for tadpoles, the importance of this effect for tadpole performance may vary depending on life stage. For example, when we ended our experiment the most advanced tadpoles were at Gosner stage 35, which is well before metamorphosis (Gosner stage 41 or above). Metamorphosis is a very energy intensive process for all tadpole species, but it is especially costly for *L. palustris* (Orlofske and Hopkins 2009). This suggests that food availability – and hence litter quantity – might have an even greater effect on *L. palustris* as individuals approach metamorphosis. Outside of food availability, it is possible that litter quantity has other important effects. For example, litter quantity may provide increase refugia from predators (Hossie and Murray 2010), which could minimize tadpole investments in costly morphological defenses and could increase tadpoles willingness to forage. Such effects would not be related to food quantity per se.

While conditions in our microcosms were sufficient for many tadpoles to survive and grow, our tadpole density (1 tadpole per L water) probably caused significant mortality and retarded tadpole development. Wilbur and Fauth (1990) found that initial tadpole density had a significant effect on whether *L. palustris* metamorphosed, and suggested that any density over 1 tadpole per 2 L of water could be stressful for this species. Importantly, conditions in our experimental units were uniformly stressful and did not preclude our ability to observe differences in tadpole performance. Microcosm and mesocosm designs may overestimate treatment effects (Skelly 2002), and this likely occurred in our experiment. We therefore suggest caution in extrapolating our results to natural pond communities. Field experiments are a logical and necessary next step towards understanding how increased litter inputs following plant invasions may impact larval amphibians.

As in our study, Rubbo et al. (2008) explicitly considered the effect of litter quantity on larval amphibian performance. They found that increasing leaf litter

quantity from native tree species was positively correlated with performance of larval *Lithobates (Rana) sylvaticus* LeConte (wood frog), but had no effect on larval salamanders (*Ambystoma* spp.). As in our study, these authors observed that dissolved oxygen decreased as litter inputs increased. Tadpole development and dissolved oxygen levels showed opposing trends in our study (dissolved oxygen decreased with litter quantity, but tadpole performance increased). Regardless of litter quantity, levels of dissolved oxygen were always greater than 5 mg/L. Studies with other larval anurans indicate that 5 mg/L is above the critical threshold where one might expect dissolved oxygen to affect tadpole behavior or performance (Wassersug and Siebert 1975, Ultsch et al. 1999); *L. palustris* tadpoles can also breathe air from the waters' surface, which may also explain their insensitivity to dissolved oxygen. Whether larger quantities of litter can force dissolved oxygen levels low enough to hinder tadpole performance remains unknown. While we did observe differences in temperature among pools, this seemed to have little effect on tadpole performance, as actual differences in temperatures were $> 1^{\circ}\text{C}$ (Figures 1.2A-C). There may have been other, unmeasured differences among pools that impacted tadpole performance, but these did not prevent us from detecting differences in tadpole performance based on experimental treatments.

Though our study and the one performed by Rubbo et al. (2008) found that *L. palustris* and *L. sylvaticus* responded positively to increased litter inputs, this may not be true of all larval amphibians. Larval amphibian responses to litter inputs could be affected by a variety of factors, including hydroperiod, competition, and predation. Many larval amphibians occupy ephemeral water bodies, and hydroperiod - or the amount of time that water remains in an area - is a key determinant of larval performance (Karraker and Gibbs 2009). Plant invasions can decrease hydroperiod in wetlands (Tuchman et al. 2009), so the negative consequences of decreased

hydroperiod on amphibians may outweigh any benefits of increased litter.

Competition and predation are two factors that strongly determine larval amphibian performance (Alford 1999). Litter inputs may alter competitive outcomes if some amphibian species benefit from increased litter more than others. Predation pressure may change with increased litter inputs; for example, litter may decrease the efficiency with which tadpole predators hunt, thereby increasing tadpole survivorship (Hossie and Murray 2010). It is unclear how an entire community of amphibian larvae may respond to changes in litter inputs, but given the responses we observed in our simplified community, we believe this represents an interesting area of future inquiry.

Empirical evidence shows that nonnative plants have similar litter chemistry compared to native plant species at the same site (Leishman et al. 2007, Leishman et al. 2010, Tecco et al. 2010), and that chemical traits, rather than plant origin, determine bottom-up energy flow in invaded ecosystems (Lecerf et al. 2007, Moline and Poff 2008, Hladysz et al. 2009, Maerz et al. 2010a). Our findings corroborate the results of these previous studies; the native and nonnative species with similar chemistry showed similar effects on our experimental food web. However, unlike many previous studies, our results also identify litter quantity as a key determinant of bottom-up energy flow in invaded ecosystems. This finding is particularly relevant to understanding the ecological impacts of plant invasions, because nonnative plants often show increased biomass production compared to the native species they replace (Ehrenfeld 2003, Liao et al. 2007, Liao et al. 2008). The reasons why nonnative plants increase plant production remain unclear; a variety of hypotheses have been proposed to explain this phenomenon, including release from natural enemies (Crawley 1987, Williamson and Fitter 1996), fluctuating resources (Davis et al. 2000), or the evolution of increased competitive ability (Blossey and Nötzold 1995), among others. Though we cannot speculate here as to why nonnative plants are so productive, we can state

that regardless of the mechanism, increased litter inputs following plant invasions can have important consequences for larval amphibians.

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CHAPTER 2

LITTER QUALITY OVERRIDES NON-CONSUMPTIVE EFFECTS OF PREDATORS IN DETERMINING TADPOLE PERFORMANCE

Abstract

Predators can have strong effects on prey, even without consuming them, but the factors that determine the magnitude and direction of these “non-consumptive effects” are not well known. We investigated whether variation in resource quality modifies non-consumptive effects of predators on prey. We assembled mesocosms where we varied plant litter quality using the grass *Phragmites australis americanus* and presence/absence of a caged predator (larval dragonfly, *Anax* spp.). Each mesocosm was stocked with an inoculum of algae, zooplankton (*Daphnia pulex*), and pickerel frog (*Lithobates palustris*) tadpoles. We predicted that as litter quality increased, the number and size of metamorphic tadpoles, the number of *D. pulex*, algal abundance, and litter mass loss would increase, while length of larval period, dissolved organic carbon and dissolved organic nitrogen would decrease. We predicted that predators would reduce the number and size of metamorphic individuals and increase length of larval period, but that these effects would attenuate as litter quality increased. As predicted, increasing litter quality had positive effects on tadpole survival, tadpole size, *D. pulex* abundance, litter mass loss, dissolved organic carbon and dissolved organic nitrogen. Contrary to our expectations, caged predators did not affect *L. palustris* performance. We found that variation in litter quality by far exceeded effects of caged predators on tadpoles. While we did not observe significant effects of predators on tadpoles, we did find some evidence for an interaction between litter quality and non-consumptive effects of predators on tadpoles. We suggest that gauging the importance of non-consumptive effects on tadpoles will be greatly

improved by re-evaluating the relationship of resource quality and non-consumptive effects through experiments employing more extensive resource quality gradients.

Introduction

Many organisms, particularly in freshwater ecosystems, rely on plant litter subsidies for energy and nutrients, consequently, changes to the quantity and quality of plant litter can have strong, cascading effects on aquatic food webs (Wallace et al. 1999, 2004, Rubbo et al. 2006, Maerz et al. 2010a, Cohen et al. 2012a, Cohen et al. 2012b). Consumer performance is not simply a function of resource quantity or quality, however, as consumers are also subject to predation. Predators can affect prey by injuring or consuming them, but they can also have non-consumptive effects (NCEs). Non-consumptive effects include any changes in prey that result from predator presence, such as changes in activity levels (Sih 1987, Lima and Dill 1990), habitat use (Werner et al. 1983, Kotler et al. 1991), or life-history (Crowl and Covich 1990, Chase 1999). For larval amphibians, empirical evidence indicates that NCEs can increase, decrease or have no effect on prey performance (Benard 2004, Bolnick and Preisser 2005, Relyea 2007). The reasons for variation in NCEs are not well understood (Benard 2004, Relyea 2007), but basal resource quality may play an important role in structuring these predator-prey interactions.

Several investigations reveal that resource quality can affect the direction and magnitude of NCEs (Danner and Joern 2003, 2004, Nicieza et al. 2006, Trussell et al. 2008, Kaplan and Thaler 2010). One line of evidence suggests that negative NCEs disappear with high quality resources, because high quality resources compensate for negative effects of predators. For example, Danner and Joern (2004) investigated NCEs of wolf spiders on grasshoppers (*Aegeneotettix deorum*) in fertilized (high quality) and unfertilized (low quality) grass plots; they found that predator risk

decreased grasshopper size and development rate in unfertilized grass, but this effect disappeared when grasshoppers fed on fertilized grass. An alternate line of evidence suggests that negative NCEs disappear with low quality resources, because prey become food-limited and begin to display 'riskier' behaviour (i.e. they increase foraging despite predator presence). Examples of this type of response exist across many taxa (Houston et al. 1993, Lima 1998, Relyea and Hoverman 2003, Kaplan and Thaler 2010). Overall, these studies demonstrate that resource quality can modify NCEs, but the nature of this relationship remains unclear.

In this study, we investigated the interactive effects of litter quality and NCEs on tadpole performance. Tadpoles are a central component in a growing body of literature demonstrating that litter subsidies strongly structure freshwater food webs (Rubbo and Kiesecker 2004, Maerz et al. 2005, Brown et al. 2006, Rubbo et al. 2006, Williams et al. 2008, Maerz et al. 2010a, Stoler and Relyea 2011, Cohen et al. 2012a, Cohen et al. 2012b). More specifically, field and mesocosm studies show that litter quality - measured in terms of carbon (C): nitrogen (N): phosphorus (P) ratios and lignin content - has substantial effects on tadpole performance (Maerz et al. 2005, Brown et al. 2006, Maerz et al. 2010a, Cohen et al. 2012a). Although the exact mechanisms are unknown, one way that litter quality may affect tadpole performance is by influencing the quality and productivity of biofilms (algae, fungi, bacteria, and protists) that tadpoles graze on. Tadpoles are also a focal organism for investigations of NCEs, as many studies have shown that caged predators affect tadpole behaviour, morphology, growth, and survival (reviewed in Relyea 2007) – our study unites these two areas of inquiry by investigating how litter quality will affect tadpole performance when predators are present.

In this study we manipulated litter quality (through litter C:N:P ratios) and NCEs (through presence/absence of caged larval dragonflies) to examine how

interactions between these forces would influence tadpole performance (measured as number of larvae reaching metamorphosis, mass at metamorphosis, and days to metamorphosis). We set up simplified ecosystems consisting of litter from *Phragmites australis americanus* Saltonstall, Peterson and Soreng (North American common reed), microbes, algae, *Daphnia pulex*, and *Lithobates palustris* LeConte (pickerel frog) tadpoles. Half of the experimental units also received a caged tadpole predator (*Anax* spp.). While the rest of our experimental organisms came from single populations, plant litter was collected from three different *P. australis americanus* stands. These populations varied in C:N:P ratios (Table 1).

We predicted that (1) tadpole performance would improve (greater number and size of metamorphic individuals, shorter larval period) as litter quality increased, (2) predators would have negative NCEs on tadpoles (decrease number and size of metamorphic individuals, increase length of larval period), and (3) that negative NCEs would attenuate as litter quality increased.

Materials and Methods

Lithobates palustris lays eggs in small, permanent water bodies such as meadow ponds and streams (Wright 1914, Pough and Kamel 1984). Relatively little is known about *L. palustris* feeding habits as larvae, but the biofilms that form on the surface of plant litter likely comprise a significant portion of their diet (Altig et al. 2007, Whiles et al. 2010). In our study region, central New York, USA, most larvae metamorphose in August and September, though overwintering is possible (Wright 1914, Pough and Kamel 1984, Wilbur and Fauth 1990). In several previous experiments, including experiments with *P. australis americanus*, we have observed that larval *L. palustris* performance is positively correlated to litter quality, particularly the C:P ratio of plant litter (Cohen 2009, Maerz et al. 2010a, Cohen et al. 2012a).

Phragmites australis americanus is a native tall perennial grass that inhabits shorelines and wetlands across much of North America (Saltonstall 2002). This endemic North American subspecies has declined greatly in the East and in many locations an invasive introduced haplotype of *P. australis* has replaced mixed wetland plant communities with near monocultures (Saltonstall 2002). The ranges of our two experimental organisms overlap widely although *P. australis* does not occur at our collection location for *L. palustris* or within its vicinity; thus *L. palustris* individuals in our experiment are likely naïve to effects of *P. australis*. Previous analyses have shown that *P. australis americanus* shows high intraspecific variation in elemental composition (C:N range 25.3 – 72.3; mean = 44.7 ± 3.54 ; N=19 populations; Martin 2010) making the species ideally suited for our investigation. Even though variation in litter quality can be much greater between species than within species, other traits (e.g. leaf structure, secondary chemistry, etc.) are likely to vary greatly between species. Working with a single species allowed us to better isolate effects of litter quality on our experimental community.

In fall 2008, we collected senesced leaves that were still attached to stems from populations in central New York (43°69'97.17", -76°18'93.00"), northern Indiana (41°27'95.56", -85°8'00.917") and northern Utah (41°71'13.06", -112°9'45.554"). We air-dried litter in mesh bags in a greenhouse under ambient temperature. In November 2008, we submitted three samples from each population for C and N (Cornell Nutrient Analysis Lab in Ithaca, NY, USA) and lignin and P (Dairy One, Ithaca, NY, www.dairyone.com) analyses. At both laboratories, leaves were ground in a ball mill (< 1 mm) before analyses. Carbon and N were measured by combustion using a Carlo Erba NC2100 Soil Analyzer (Thermoquest, Italy). Phosphorus was measured using near infrared reflectance spectroscopy using a Foss NIRSystems Model 6500 (FOSS NIRSystems, Inc, Laurel, MD, USA). Lignin content was measured by filter bag

technique; samples were placed in ANKOM (Ankom Technology, Macedo, NY, USA) filter bags and digested in 2 L acid detergent fibre solution in an ANKOM A200 Digestion Unit. Samples were kept in filter bags and rinsed three times in boiling water, then rinsed in acetone and dried at 100°C for 2 hours. Residues were digested in 72% sulphuric acid for 3 hours in an ANKOM Daisy^{II} Incubator at ambient temperature.

In May 2009, we established experimental ecosystems in 100 L plastic pots (BFG supply, Lancaster NY) at the Cornell Resource Ecology and Management Facility in Ithaca, NY, USA on an open, level area. We employed a 3 x 2 factorial design (N=20 replicates per treatment), with three levels of litter quality (low, medium and high; Table 1) and presence or absence of a caged predator. We placed mesocosms (N=120) in 10 rows of 12 and randomly assigned treatments. We covered mesocosms with a screened lid (2 mm mesh) to prevent colonization by other organisms. We filled each mesocosm with 80 L of tap water on 15 May 2009, and added 20 g of litter on 17 May 2009 from one of three *P. australis americanus* populations (40 mesocosms per litter source). On 20 May 2009, we inoculated each mesocosm with 0.5 L of filtered pond water (80 µm mesh) from the Cornell Experimental Ponds in Ithaca, NY USA. We evenly spaced three tiles (3 x 3 cm) on the bottom of each mesocosm to collect periphyton, which we later used to measure algal abundance. On 25 May 2009, we collected *D. pulex* from the Cornell Experimental Ponds and added 20 adult individuals to each mesocosm. *Daphnia pulex* were not a focal organism in this experiment, we added them simply to better mimic the tadpoles' natural environment, as is commonly done in experiments of this kind (Rittenhouse 2011).

On 06 May 2009, we collected five *L. palustris* egg masses (500-1,000 eggs per mass) at the Arnot Forest in Van Etten, NY, USA. We kept individual egg masses

in coarsely filtered pond water in gauze-covered 15 L plastic containers until eggs hatched on 16 May 2009. All containers floated in a small outdoor pool to prevent large and rapid temperature fluctuations. After hatching, we fed tadpoles fish flakes *ad libitum* and changed water as needed. On 01 June 2009, we added ten tadpoles (two from each egg mass) to each mesocosm. We chose this density because a previous experiment with *L. palustris* suggested that it would allow for strong intraspecific competition without precluding metamorphosis (Wilbur and Fauth 1990).

To manipulate predator cues we placed a single cage (pvc cylinder, 17.6 cm diameter, 10 cm height, enclosed in 1 mm mesh) into each mesocosm. The cage was designed to allow dissipation of chemical cues from caged predators, though it is possible tadpoles could also see predators through the 1 mm mesh at the top of the cylinder. We chose dragonfly larvae because they are the most common predator used in experiments that assess NCEs on tadpoles (Relyea 2007). We collected dragonfly larvae (*Anax* sp.) from the Cornell Experimental Ponds, Ithaca, NY and housed them in outdoor mesocosms with American toad (*Anaxyrus americanus*) or green frog (*Lithobates clamitans*) tadpoles for several days before adding them to mesocosms. We regularly observed dragonfly larvae preying upon tadpoles in these ‘holding’ mesocosms. On 02 June 2009, we added a single dragonfly larvae to cages in the appropriate mesocosms. We assumed that one dragonfly larvae per mesocosm would be sufficient to generate NCEs because a previous study demonstrated NCEs on tadpoles from a single dragonfly larvae in tanks ten times the size of ours (Peacor 2002). We checked on dragonfly larvae every two days and replaced dead individuals or those that had been in containers for more than a week. We also “checked” those mesocosms without predators by lifting the empty cage out and replacing it, in order to minimize differences between treatments with and without predators (e.g. effects due

to repeated disturbance). We collected new dragonfly larvae from the Cornell Experimental Ponds as needed.

We measured dissolved oxygen (mg L^{-1}), pH, and temperature ($^{\circ}\text{C}$) every other week using a YSI 556 MPS meter (YSI Environmental Monitoring, Yellow Springs, OH) in each mesocosm. In addition, we took 50 mL samples from the middle water column in June and July and measured dissolved organic carbon (DOC) and nitrogen (DON) on a Shimadzu 5050 analyzer (Shimadzu Corporation, Kyoto, Japan) after adjusting pH to 2.5 and purging with CO_2 -free N_2 . We also randomly removed one tile from each mesocosm monthly from June-August. We stored samples and measured algal abundance (chlorophyll-a) following Steinman et al. (2006). On 13 August 2009, we sampled 1 L from the middle water column of each mesocosm and filtered out all *D. pulex*. We preserved *D. pulex* in 90% ethanol and counted them during fall 2009.

We checked our mesocosms for metamorphs twice weekly until the first *L. palustris* metamorph appeared on 22 July 2009. Each day thereafter, we checked mesocosms twice daily and removed *L. palustris* with at least one forelimb erupted (stage 42 or above, Gosner 1960). We placed metamorphs in individual 250 mL cups with a moist paper towel until they completely resorbed their tails (1-3 days). We then weighed them and measured their snout-vent length. We terminated the experiment on 27 September 2009, 118 days after we first added tadpoles, when a week had elapsed without new metamorphs and we had not observed any remaining individuals that were close to metamorphosis (Gosner stage 40 or above). We collected all remaining tadpoles and measured their snout-vent length and Gosner stage. The following day, we collected all remaining plant litter and dried it at 60°C for 72 hours before weighing it.

We measured tadpole performance using three common metrics: number of tadpoles that metamorphosed, mass at metamorphosis, and time to metamorphosis (the number of days between tadpole hatching and completing metamorphosis, e.g. Rubbo and Kiesecker 2004). We used these metrics because larval amphibians that complete metamorphosis earlier and at a larger size generally have higher fitness compared to conspecifics that metamorphose later and at a smaller size (Smith 1987, Semlitsch et al. 1988, Berven 1990). In addition, we chose these metrics because previous experiments have used them to assess NCEs from tadpole predators (reviewed in Relyea 2007). We used MANOVA and ANOVA to examine variation in tadpole performance among treatments. We combined number of tadpoles that metamorphosed, metamorph mass, and time to metamorphosis into a multivariate response variable in a MANOVA, where litter quality, caged predator (present or absent) and the interaction between these were fixed effect variables. To interpret patterns revealed by MANOVA, we then conducted a series of Bonferroni-adjusted ANOVAs with each of our performance metrics as individual response variables. The significance level for these ANOVAs was 0.017.

We performed individual ANOVAs with percent dissolved oxygen, pH, temperature, June and July DOC, June and July DON, litter mass loss, algal abundance, and *D. pulex* abundance as individual response variables; in each case we used litter quality, predator (present or absent) and the interaction between these factors as fixed effect variables. We applied post-hoc tests (Tukey's HSD) following all ANOVAs when appropriate. We applied an arcsine square root transformation to count data and log-transformed all other response variables to ensure that assumptions of normality and homogeneity of variance were met.

Results

Overall, our results show that litter quality affected tadpole performance, while predators had little effect. Of the 1200 tadpoles we added to our mesocosms, 594 (49.5%), survived to the end of the experiment and 274 (22.8%) completed metamorphosis. The mean mass at metamorphosis was 0.66 g (± 0.031 SE), but there was a considerable size range (0.38 – 1.80 g). On average, tadpoles took 84.3 days (± 0.88 SE) to complete metamorphosis. In the MANOVA analysis, where number of tadpoles that metamorphosed, mass at metamorphosis, and time to metamorphosis were taken together as a multivariate response, we found a significant effect of litter quality (Wilks' $\lambda = 0.76$, $F_{6,180} = 4.38$, $p < 0.001$), but predator presence and the interaction between litter quality and predator presence had no effect. In the individual Bonferroni-adjusted ANOVAs, litter quality was the only factor that had a significant effect on number of tadpoles that metamorphosed ($MS = 0.2$, $F_{2,86} = 8.8$, $p < 0.001$, Figure 2.1A); significantly more tadpoles reached metamorphosis in mesocosms with high and medium quality litter compared to low quality litter. Litter quality also affected mass at metamorphosis ($MS = 0.39$, $F_{2,86} = 4.57$, $p = 0.012$, Figure 2.1B); *L. palustris* metamorphs were significantly larger when raised with high quality litter compared to low quality litter. None of the factors we tested had a significant effect on time to metamorphosis (Figure 2.1C).

Abiotic factors varied little among pots, and all measures indicated that conditions in mesocosms were conducive to tadpole development (Ultsch et al. 1999). Across all mesocosms, the mean dissolved oxygen was 7.2 mg L⁻¹ (± 0.08 SE), mean pH was 8.67 (± 0.02 SE), and mean temperature was 22.5 °C (± 0.12 SE). Dissolved oxygen was significantly higher in mesocosms with low quality litter (ANOVA, $MS = 8.84$, $F_{2,108} = 12.8$, $p < 0.001$), with mean dissolved oxygen ranging from 7.7 mg L⁻¹ (± 0.21) for low quality litter to 6.8 mg L⁻¹ (± 0.09 SE) for high quality litter. There was no significant difference in pH or temperature across treatments. In June, mean DOC

was 6.6 mg L^{-1} ($\pm 0.21 \text{ SE}$) and mean DON was 0.61 mg L^{-1} ($\pm 0.027 \text{ SE}$), while in July mean DOC was 9.5 mg L^{-1} ($\pm 0.20 \text{ SE}$) and mean DON was 0.58 mg L^{-1} ($\pm 0.050 \text{ SE}$). In July, DOC varied significantly based on litter quality (ANOVA, $MS = 31.7$, $F_{2,117} = 8.13$, $p < 0.001$, Figure 2.2A), with more DOC in low quality litter compared to medium and high quality litter. Litter quality had a significant effect on DON in June (ANOVA, $MS = 0.899$, $F_{2,117} = 14.8$, $p < 0.001$, Figure 2.2B); there was significantly more DON in low quality litter compared to medium and high quality litter. June DOC and July DON did not vary significantly among treatments. Algal abundance averaged $10.5 \mu\text{g L}^{-1}$ ($\pm 0.87 \text{ SE}$) and did not vary significantly among treatments. The mean number of *D. pulex* per mesocosm was $95.2 \text{ individuals L}^{-1}$ ($\pm 9.82 \text{ SE}$), but there was a considerable range across mesocosms ($0 - 612 \text{ individuals L}^{-1}$). Litter quality was the only factor that had a significant effect on *D. pulex* abundance (ANOVA, $MS = 6.7$, $F_{2,117} = 5.9$, $p = 0.004$, Figure 2.3). Mesocosms with high and medium quality litter had significantly more *D. pulex* compared to mesocosms with low quality litter.

The amount of litter mass loss by the end of the experiment varied significantly based on litter quality ($MS = 124$, $F_{2,117} = 25.2$, $p < 0.001$), and each litter population was significantly different from all others. Interestingly, in the litter populations we selected for this experiment, lignin content was inversely related to C:N:P ratios, and all litter traits we measured were highly correlated. For lignin and N, the Pearson product-moment correlation, r , was 0.96 ($p < 0.001$), for lignin and P, $r = 0.99$ ($p < 0.001$), and for N and P, $r = 0.91$ ($p < 0.001$). The litter with the highest % N and % P also had the highest lignin content, such that litter with the highest N and P content lost the least amount of mass after 131 days. It appears that mass loss was strongly influenced by initial lignin content ($r = 0.30$, $p < 0.001$, Figure 2.4). Predator presence had no effect on litter mass loss.

Table 2.1 Litter chemistry for three *P. australis americanus* populations used as basal resource in experimental communities.

Litter quality	%C	% N	% P	C:N	C:P	N:P	% Lignin
High	45.8	1.51	0.09	30.5	509	16.8	10.5
Medium	42.5	1.36	0.05	31.2	849	27.2	7.1
Low	41.0	0.99	0.03	41.3	1370	33.1	4.3

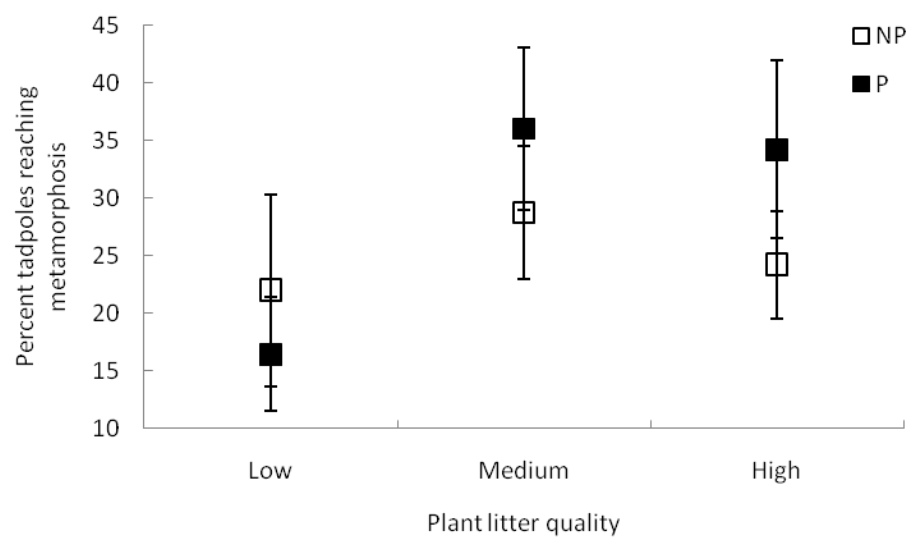


Figure 2.1A Number of *Lithobates palustris* reaching metamorphosis (open symbols/NP are replicates without a caged predator, filled boxes/P replicates with a caged predator). Data are means ± 2 SE of 10 replicates per treatment.



Figure 2.1B *Lithobates palustris* mass at metamorphosis (open symbols/NP are replicates without a caged predator, filled boxes/P replicates with a caged predator). Data are means \pm 2 SE of 10 replicates per treatment.

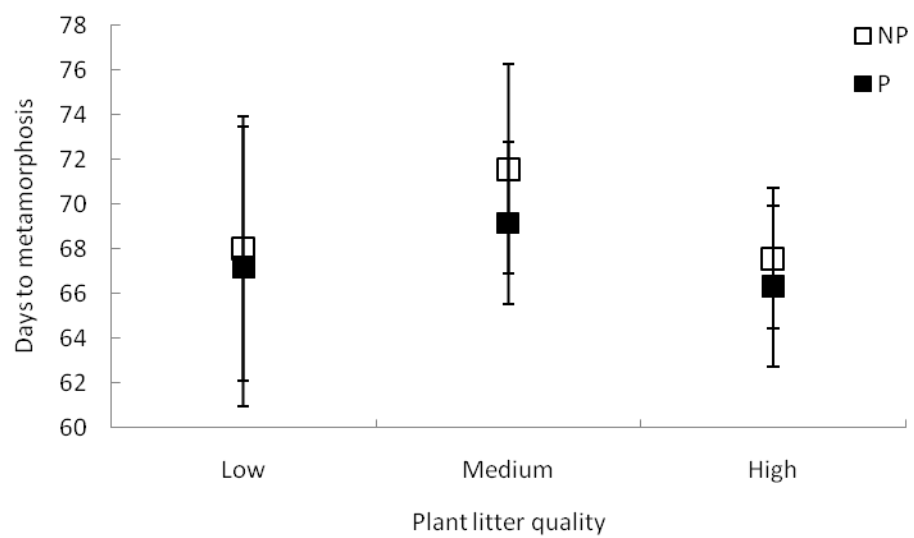


Figure 2.1C *Lithobates palustris* days to metamorphosis (open symbols/NP are replicates without a caged predator, filled boxes/P replicates with a caged predator). Data are means ± 2 SE of 10 replicates per treatment.

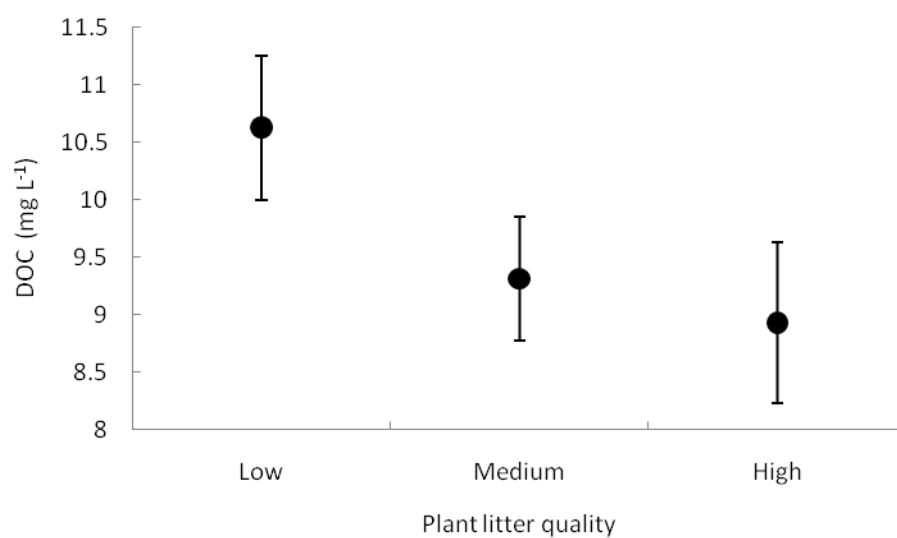


Figure 2.2A Effects of litter quality on dissolved organic carbon (DOC). Circles show means across all treatments for each level of litter quality and error bars represent ± 2 SE of 10 replicates per treatment.

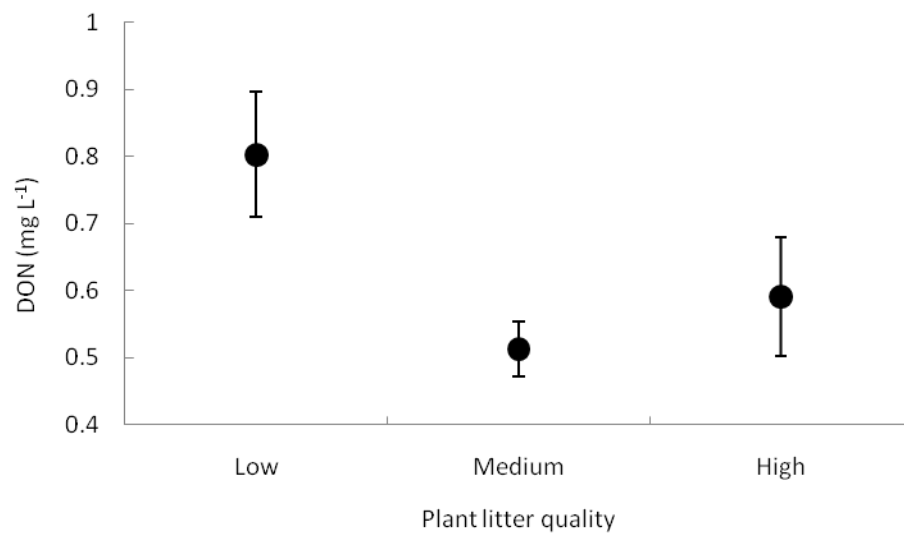


Figure 2.2B Effects of litter quality on dissolved organic nitrogen (DON). Circles show means across all treatments for each level of litter quality and error bars represent ± 2 SE of 10 replicates per treatment.

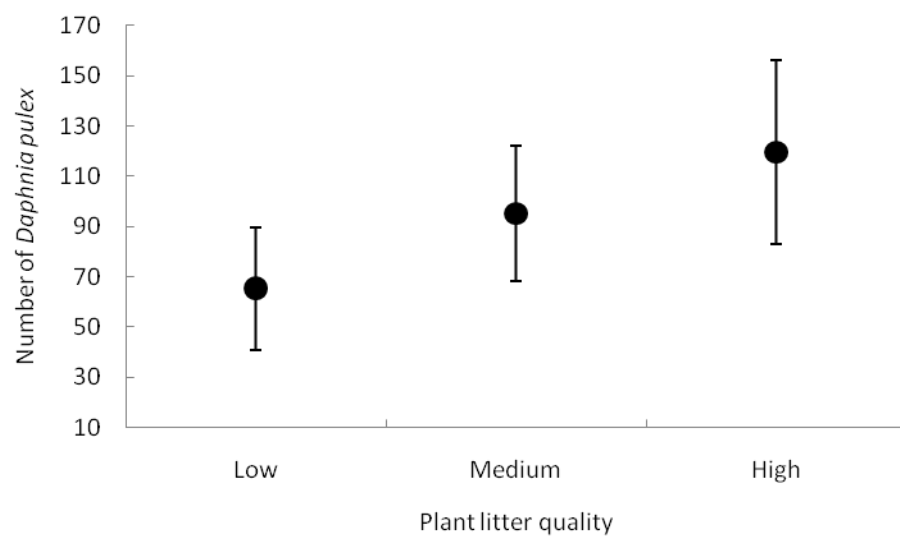


Figure 2.3 Effects of litter quality on number of *Daphnia pulex*. Circles show means across all treatments for each level of litter quality and error bars represent ± 2 SE of 10 replicates per treatment.

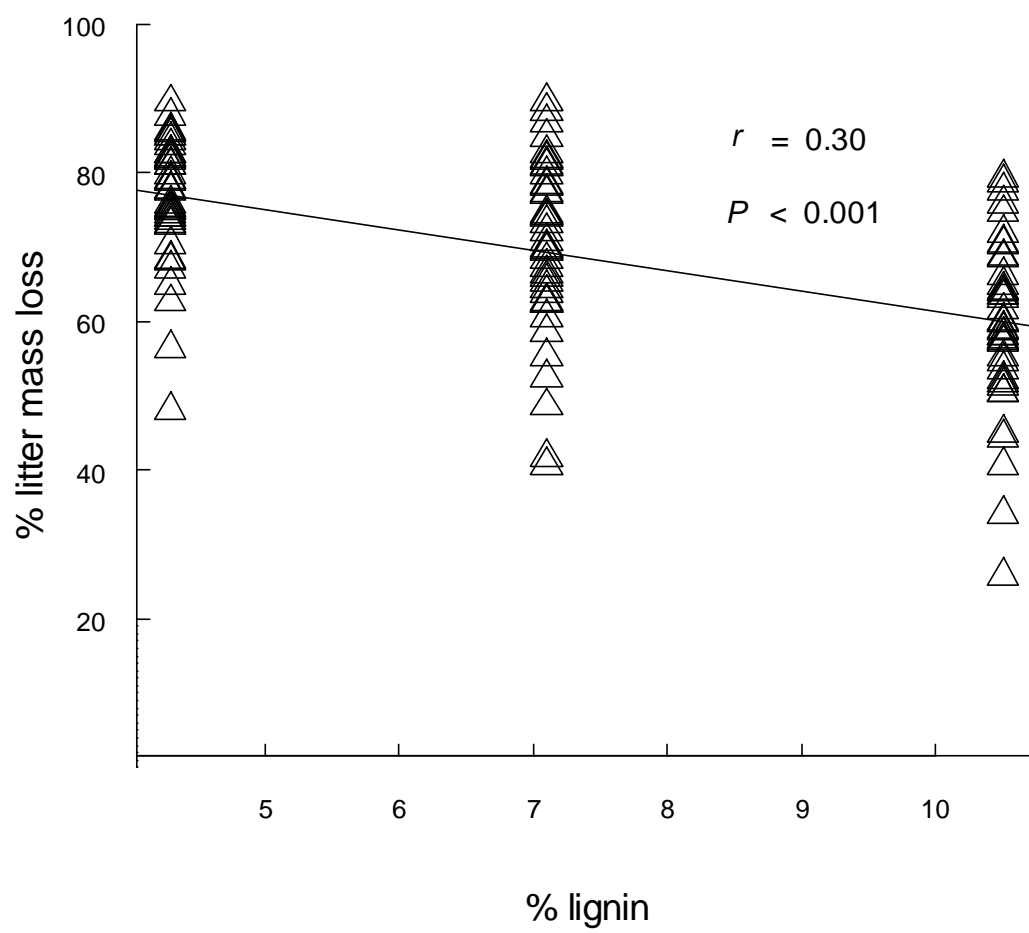


Figure 2.4 Litter mass remaining as a function of litter lignin content.

Discussion

We used a gradient of litter quality to examine how variation in basal resource quality interacts with NCEs to determine tadpole performance. Our results support our first prediction, that tadpole performance would improve with increased litter quality, as the number of metamorphs and the mass of individual metamorphs increased with litter quality. In this respect, our results agree with previous studies that find a positive relationship between litter quality and larval amphibian performance (Maerz et al. 2005, Brown et al. 2006, Williams et al. 2008, Maerz et al. 2010a, Cohen et al. 2012a). Contrary to our second and third predictions, however, we did not observe NCEs on tadpoles and did not observe a statistically significant interaction between litter quality and NCEs.

To the best of our knowledge, our study is the first to examine interactions between litter quality and NCEs, and we find that litter quality is the driving force in our community with little or no effects of caged predators. Effects of litter quality permeated nearly every abiotic and biotic parameter that we measured in our experiment, from the amount of DOC to *L. palustris* mass at metamorphosis. This clearly shows the primacy of basal resource quality as an ecological driver in our system.

We cannot exclude the possibility that NCEs occurred in our experiment but that we failed to detect them. This could potentially include effects of predators on elements of tadpole performance that we did not measure, such as tail morphology (Schoeppner and Relyea 2009b) or post-metamorphic performance (Nicieza et al. 2006). However, we were interested in assessing NCEs with ecological relevance on tadpole survival, development time and metamorph size and these important metrics did not respond significantly to predator cues.

We also acknowledge the possibility that predator cues in our design were not strong enough to change *L. palustris* performance, however we think this is unlikely for several reasons. First, a lack of apparent NCEs could be a consequence of the large size of our mesocosms diluting the signal of a single *Anax* (Peacor 2006). However, similar experiments have found NCEs from larval dragonflies on tadpoles in tanks ten times the size of ours (e.g. Peacor 2002). Moreover, we ran the experiment from May to September, continually replacing *Anax* along the way. Tadpoles in predator treatments were repeatedly exposed to chemical cues, so cues were frequent, if not entirely constant. Trussell et al. (2011) examined whether predator risk must be constant in order to incite NCEs and found that short, episodic pulses of predator risk influenced consumer growth and resource acquisition nearly as much as constant exposure. Second, while previous work has shown that tadpoles respond most strongly to cues if conspecifics are used as prey for caged *Anax* (Schoeppner and Relyea 2005, 2009c) they still respond if *Anax* larvae are fed other amphibian species (Schoeppner and Relyea 2009a, c), as they were in this experiment. Third, our experimental design permitted dynamic feedbacks between *L. palustris* and their resources, as we did not regulate resource levels within mesocosms - this is in contrast to a 'static resource' design, where a constant supply of resources is maintained throughout the experiment (Preisser et al. 2009). NCEs are stronger in dynamic systems like ours (Preisser et al. 2009), so our dynamic resource design makes it more likely that we would detect NCEs on tadpoles – if there had been any. For all of these reasons we consider it unlikely that we failed to detect NCEs because of a flaw in our experimental design.

Caged predators could have affected tadpoles in ways beyond scaring them. For example, it is possible that predators benefited tadpoles by excreting nutrients, thereby increasing periphyton growth (Anholt et al. 2000). Our data show no evidence

for this, as predators had no effect on litter mass loss, DOC, DON, algae, or *D. pulex*. It is unlikely that nutrient inputs from predators would affect tadpoles without affecting these other components of the ecosystem. In addition, Peacor (2002) reported that several experiments found no significant effect of caged larval dragonflies on periphyton growth in aquatic mesocosms, a result we also observed here. Overall, predator excretion appears to have had negligible effects on tadpole performance, though it may show an effect in much smaller mesocosms, as Anholt et al. (2000) observed.

Interestingly, although NCEs were not statistically significant, there was a tendency for predators to exacerbate effects of litter quality on tadpoles. For example, when no predators were present, high quality litter increased the mean number of *L. palustris* metamorphosing by 10% compared to low quality litter. However, in mesocosms with a caged predator, the mean number of metamorphs in mesocosms with high quality litter was 108% higher than in mesocosms with low quality litter. Similarly, compared to mesocosms with low quality litter, high quality litter increased mean metamorph mass by 8.8% when no predators were present, but raised it by 57.5% when a caged predator was present. The large variation among our replicates in different treatments resulted in non-significant differences between our three litter treatments, but we cannot exclude the possibility of significant NCEs under a wider range of litter quality in *P. australis americanus*. The known range of litter quality in this species is larger than what we found in the populations used in this experiment; others have observed a range in C:N ratios of 25.3-72.3 (Martin 2010). Moreover, for a previous experiment, we measured litter quality for nine plant species common to wetlands in New York, USA, including *P. australis americanus*, and found that C:N ranged from 14.3 – 124.1 (Cohen et al. 2012a), a range ten times greater than what we observed here, while C:P ranged from 6031.8 – 172.3 (Cohen et al. 2012a), which is

seven times greater than the range observed in this experiment. Thus tadpoles are likely to encounter a wide range of litter quality in natural environments, and this wider range may be large enough to affect NCEs from tadpole predators.

Elemental ratios, specifically C:N:P ratios, strongly structure species interactions (Sternner and Elser 2002). In our experimental ecosystem, litter C:N:P ratios influenced myriad biological properties, and had a greater influence on prey performance than NCEs from predators. Our study demonstrates that effects of litter quality persist or even magnify as communities become more complex and we strongly advocate for additional experiments to assess the importance of this variation in basal resource quality on NCEs.

Our results point towards the need to further investigate how the huge variation in predation pressure and basal plant resource quality are likely to interact in determining tadpole performance in more complex environments. Our experiment is the first to combine predator cues with variation in litter quality in the way most tadpoles in their natural environments would actually encounter them – together. Studying each in isolation is prone to overemphasize effects of the one under investigation. We assessed the magnitude of basal resource quality and NCE effects on biologically relevant performance indicators in tadpoles and *Daphnia* as well as other components of the food web. Thus, our design and analyses are more complex and complete than many other experiments. That we fail to find NCEs with relevance for species performance is an indication that they may not be as important as previous work suggests. We urgently need further and more detailed studies to gauge the effect of both resource quality gradients and NCEs on a wide range of taxa to assess the generality and magnitude of NCEs. If our data exemplify what could be found in other studies that combine these two factors, the importance of NCEs in structuring food webs may need to be re-evaluated.

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CHAPTER 3

NO APPARENT EFFECTS OF SOIL BIOTA ON GREEN FROG TADPOLE PERFORMANCE

Abstract

Invasions by nonnative plant species are transforming plant communities across the globe. An important challenge for ecologists to understand how animals will respond to these changes. One way that plant invasions could affect aquatic animals is by changing the rate at which soil communities decompose litter, which could alter the flow of energy and nutrients from plant litter to aquatic communities. In this study, we measured larval amphibian responses to soil conditioned by either introduced or native genotypes of *Phragmites australis* L. (common reed) in northeastern North America. We collected soil from adjacent stands of introduced and native *P. australis* at three sites in central New York, and inoculated outdoor aquatic mesocosms with soil extracts. Mesocosms contained six *Lithobates clamitans* Latreille (green frog) tadpoles and either low or high quality native *P. australis americanus* litter. We found that litter decomposition differed based on soil inoculum, and we observed a significant interaction between litter quality and soil inoculum; higher quality litter tended to decompose faster when exposed to inocula from introduced *P. australis americanus*, while lower quality litter tended to decompose faster when exposed to inocula from native *P. australis*. Tadpoles raised with high quality litter developed faster and achieved greater body size, but soil inocula had no apparent effect on tadpoles. Our results suggest that plant invasions may alter microbial communities, causing subtle changes in litter decomposition rates, but these changes do not appear strong enough to influence larvae of a widespread amphibian.

Introduction

The spread of introduced plants is transforming ecosystems from mixed communities of native plant species into near monocultures. Apart from the obvious visual transformation, the process begs the question whether other trophic levels are affected, and if so, what are the mechanisms responsible for these impacts (Levine et al. 2003). Plant litter is a key energy subsidy to aquatic ecosystems (Wetzel 1995, Wallace et al. 1997) and several studies show that plant invasions can affect tadpoles by changing the quality (Maerz et al. 2010a, Cohen et al. 2012a) and quantity (Cohen et al. 2012b) of litter inputs.

The quantity and quality of litter certainly influence plant decomposition – and hence, energy flow to tadpoles – but there is a growing appreciation that the decomposer community also plays an important role in this process (Strickland et al. 2009, Bardgett and Wardle 2010). For years, scientists considered decomposers to be functionally redundant, assuming that it did not matter *who* was doing the decomposing, only *what* was decomposing and *where* (i.e. under what conditions). However, several studies show that changes in microbial community structure can alter rates of litter decomposition. It remains to be seen whether differences in microbial communities can ultimately influence energy flow to aquatic consumers.

Plant invasions often increase rates of litter decomposition (reviewed by Liao et al. 2008, but see Godoy et al. 2010), and recent research suggests that changes in microbial communities may explain why (Holly et al. 2009). In this study, we examined whether establishment of introduced European genotypes of *Phragmites australis* (common reed, Saltonstall 2002), a widespread wetland plant invader (for a synopsis of documented and suspected ecosystem impacts see Marks et al. 1994, Weis and Weis 2003, Meyerson et al. 2008), would affect the function of wetlands as larval habitat for a widespread North American amphibian (*Lithobates clamitans* Latrielle,

green frog). We used native genotypes of *P. australis* – *P. australis americanus* – as a reference plant species. We purposefully excluded any spatial, structural and associated effects (e.g. changes in temperature, moisture, prey availability) created by extensive monocultures of this tall grass. Instead, we focused on potential effects of local microbial communities conditioned by either native or introduced *P. australis* on larval amphibian performance. Soil conditioning occurs when plants select for particular soil communities; these soil communities may carry out processes in a way that is favorable to the ‘parent’ plant, creating positive plant-soil feedbacks (Bardgett and Wardle 2010) but the reverse is also possible (Bever et al. 2010). For invasive plants, positive feedbacks often include accelerated rates of nutrient cycling (e.g. rapid decomposition, Allison and Vitousek 2004).

We decided to compare soil communities conditioned by introduced *P. australis* to those conditioned by native *P. australis* because native and introduced genotypes have different ecophysiological strategies; we therefore expected them to harbor different microbial communities. Introduced *P. australis* attains greater photosynthetic rates, greater biomass, and greater % nitrogen (N) compared to native *P. australis* growing at the same site (Mozdzer and Zieman 2010), and nutrient additions have a disproportionately positive effect on introduced *P. australis* growth compared to native genotypes (Holdredge et al. 2010), indicating that introduced genotypes flourish in environments with high levels of nutrients. Moreover, introduced *P. australis* accelerates N cycling rates compared to native *Spartina patens* (Windham and Ehrenfeld 2003, Windham and Meyerson 2003). Ongoing comparisons of microbial community composition and function between native and introduced *P. australis* by Crocker and Nelson (pers. communication, unpublished data; focusing on soil pathogens) show that when compared to native genotypes, introduced *P. australis* generates distinct microbial communities. We expect that

these communities specialize in rapid nutrient cycling, including accelerated rates of litter decomposition.

Our experimental design consisted of simplified pond communities containing plant litter, microbes, algae and tadpoles. We obtained plant litter from two populations of native *P. australis americanus* that differed in litter quality (Table 1); we define “higher quality” litter as litter with lower ratios of carbon (C): nitrogen (N) and C: phosphorus (P), as well as lower lignin content. To assess soil legacy effects of dominant plants (assuming but not quantitatively assessing changes in microbial communities), we created soil inocula from soils collected at six different stands (3 each of native and introduced *P. australis* genotypes). We predicted that (1) higher quality litter would decompose faster than lower quality litter; (2) litter would decompose faster when exposed to soil inocula conditioned by introduced *P. australis*, and (3) that faster decomposing litter would increase tadpole survival and development rate. To the best of our knowledge this is the first assessment integrating soil legacy effects, plant invasions, litter quality, and amphibian performance into a single study.

Materials and Methods

During November 2009, we collected senesced, but not abscised *P. australis* leaves at six sites to assess differences in litter quality (see below). We used these same sites, which have long-established *P. australis* patches (known by BB for >15 years), to collect soil samples. In addition, we collected larger litter quantities of *P. australis americanus* in northern Utah (41.711306, -112.945554) and northern Indiana (41.279556, -85.800917), two sites with substantial differences in litter quality (Martin 2010, Table 1). We air-dried litter in mesh bags in a greenhouse under ambient temperature. In December 2009, we submitted four samples from each of our 8

populations for carbon and nitrogen (Cornell Nutrient Analysis Lab, Ithaca, NY, USA) and lignin and phosphorus (Dairy One, Ithaca, NY, www.dairyone.com) analyses. At both laboratories, leaves were ground in a ball mill (< 1 mm) before analyses. Carbon and N were measured by combustion using a Carlo Erba NC2100 Soil Analyzer (Thermoquest, Italy). Phosphorus was measured using near infrared reflectance spectroscopy using a Foss NIRSystems Model 6500 (FOSS NIRSystems, Inc, Laurel, MD, USA). Lignin content was measured by filter bag technique; samples were placed in ANKOM (Ankom Technology, Macedo, NY, USA) filter bags and digested in 2 L acid detergent fiber solution in an ANKOM A200 Digestion Unit. Samples were kept in filter bags and rinsed three times in boiling water, then rinsed in acetone and dried at 100°C for 2 hours. Residues were digested in 72% sulfuric acid for 3 hours in an ANKOM Daisy^{II} Incubator at ambient temperature.

On 24 May 2010, we collected surface soil (< 10 cm depth) from three wetlands each containing distinct and spatially separated patches of both native *P. australis americanus* and introduced *P. australis*. We collected 2 kg at each wetland (1 kg from soil dominated by native *P. australis* and 1 kg from soil dominated by introduced *P. australis*), for 6 soil samples in total. We homogenized and sieved soil (2 mm) from each patch separately, then created a liquid ‘soil inoculum’. To create each soil inoculum, we added 500 g of oven-dry weight equivalent soil (Jarrell et al. 1999) to 10 L of aged tap water and let the mixture stand in a closed container for one week.

Our experiment was conducted in outdoor mesocosms (100 L tree pots) outfitted with screened (2 mm) lids. We arranged 120 mesocosms on a level surface in 10 rows of 12 and on 26 May, added 80 L of tap water and 25 g of native *P. australis americanus* leaf litter according to treatment (low or high litter quality) to each mesocosm. On 1 June 2010, we filtered the suspension (11 µm) of our soil

inoculum and immediately added 250 mL to outdoor mesocosms according to treatment. We replicated each treatment 10 times (6 soil inocula x 2 litter types x 10 replicates = 120 total mesocosms) and distributed treatments randomly among mesocosms.

On 3 June we collected six *L. clamitans* egg masses (estimated 1,000 – 2,000 eggs/mass) from several ponds in Ithaca, New York, USA. We placed egg masses individually into 15 L plastic containers with filtered (1 mm pore size) pond water, and floated containers in a shaded outdoor pond until eggs hatched (10-12 June). We fed tadpoles fish flakes *ad libitum* and on 16 June, we added six tadpoles (1 from each egg mass) at Gosner (1960) stage 26 to each mesocosm. We chose this density because it fit within the range used in previous studies (Peacor and Werner 2000, Relyea and Yurewicz 2002, Relyea 2006, Werner and Peacor 2006).

We measured dissolved oxygen, pH, and temperature in mesocosms every two weeks using a YSI 556 MPS meter (YSI Environmental Monitoring, Yellow Spring, Ohio, USA), as these parameters are known to influence tadpole performance (Ultsch et al. 1999). Beginning in mid-July, we inspected mesocosms daily for signs of metamorphic (Gosner stage 41 or higher) *L. clamitans*. We observed an individual at Gosner stage 41 on July 27. At this time, we removed all remaining tadpoles from each mesocosm to allow determination of developmental rates and recorded Gosner stage and wet mass (0.01 g) of all tadpoles. On 8 August 2010, we collected remaining litter from pots, dried litter at 60 ° C for 72 hours and immediately weighed it to the nearest 0.01 g.

We calculated litter percent mass loss as:

$$(1 - [\text{final dry mass} / \text{initial dry mass}]) * 100$$

We used two-way analysis of variance (ANOVA) to test for differences in litter mass loss based on litter type and soil inoculum source. For each of the three wetland sites,

we performed planned comparisons to assess whether litter exposed to soil inocula collected from introduced *P. australis* decomposed at a different rate compared to litter exposed to inocula collected under native *P. australis americanus*. We performed these comparisons for both litter types, and assumed a significance level of 0.05 for each comparison. We performed a MANOVA with tadpole Gosner stage and body mass as response variables, followed by individual Bonferroni-corrected ANOVAs. We then performed post-hoc tests (Tukey's HSD) where appropriate. We used an arcsin-square root transformation on litter mass loss data, and log-transformed Gosner stage and body mass to ensure that assumptions of normality were met.

Results

Soil inoculum and the interaction between soil inoculum and litter quality affected litter decomposition. Amphibian performance responded to litter quality but showed no response to soil inoculum. For the three wetlands where we collected soil inocula, native *P. australis* had higher ratios of C:N and C:P, but lower % lignin compared to litter from introduced *P. australis* (Table 3.2). Differences in litter mass loss between high and low quality litter types were marginally significant ($F = 3.07$, $df = 1$, $P = 0.08$, Figure 3.1). Soil inoculum source had a significant effect on litter mass loss ($F = 3.96$, $df = 5$, $P < 0.01$, Figure 3.1). We also found a significant interaction between litter type and soil inoculum in determining litter mass loss ($F = 3.98$, $df = 5$, $P < 0.01$, Figure 3.1). There was a general trend for low quality litter to decompose faster when exposed to inoculum from native *P. australis*, while high quality litter decomposed faster when exposed to inoculum from introduced *P. australis americanus* (Figure 3.1). Planned comparisons revealed that for soil inoculum collected at site B, lower quality litter decomposed faster when exposed to soil inoculum from native *P. australis* than when it was exposed to soil inoculum from introduced *P. australis* ($t =$

3.03, $df = 16.40$, $P < 0.01$, Figure 3.1). For soil inoculum collected at site A, higher quality litter decomposed faster when exposed to soil inocula from native *P. australis* than when it was exposed to soil inocula from introduced *P. australis* ($t = -4.66$, $df = 17.63$, $P < 0.01$, Figure 3.1), none of the other planned comparisons were significant. Contrary to our expectations, we did not observe a pattern in the way soil communities responded to litter based on the chemistry of their ‘home’ litter (Figure 3.2).

Larval *L. clamitans* survival was high (96% across all mesocosms), and did not vary significantly among soil inoculum treatments. Instead, MANOVA results indicated that *L. clamitans* performance was best explained by differences in litter quality (Wilks’ $\lambda = 0.91$, $F_{2,107} = 5.55$, $P = 0.005$). Individual ANOVAs revealed that tadpole Gosner stage was significantly greater ($F_{1,108} = 7.25$, $P = 0.008$) and body mass significantly greater ($F_{1,108} = 10.8$, $P = 0.001$) with high quality litter as the basal resource (Figure 3.3).

Table 3.1 Litter quality metrics for two populations of native *Phragmites australis americanus* litter used as a basal resource in mesocosms.

Litter quality	C:N	C:P	% Lignin	Location
Low	42.7	1322.7	7.4	Utah
High	41.0	772	6.0	Indiana

Table 3.2 Litter quality metrics for *P. australis* litter where soil inocula was collected

Site	Origin	Lignin		
		(mg/g)	C:N	C:P
A	Native	6.9	29.9	629.9
	Introduced	7.9	28.6	468.4
B	Native	5.4	22.1	346.9
	Introduced	9.4	22.7	378.3
C	Native	10.7	31.3	501.1
	Introduced	12.1	26.7	242.4

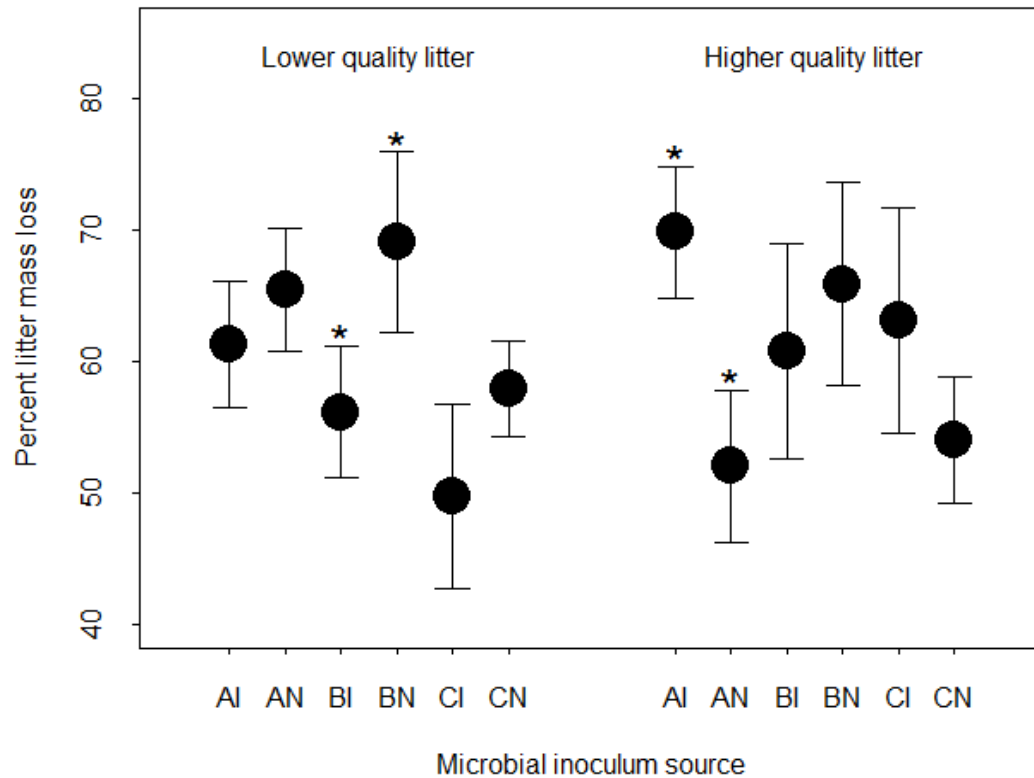


Figure 3.1 Litter mass loss (%) as a function of *Phragmites australis americanus* litter quality (high or low) and soil inoculum source (3 sites A-C; each with a patch of native (N) or introduced (I). Data are means \pm 2 SE of 10 replicates per treatment. Asterisks denote significantly different pairs ($P < 0.05$).

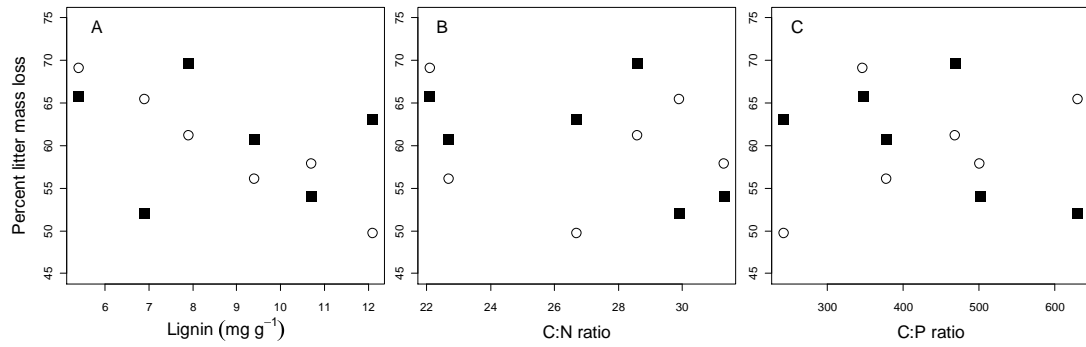


Figure 3.2 Litter mass loss (%) as a function of gradients in *P. australis* litter traits (lignin; C:N; C:P) at source populations for soil inocula. Circles represent low and squares represent high quality litter. Data are means of 10 replicates for each litter quality.

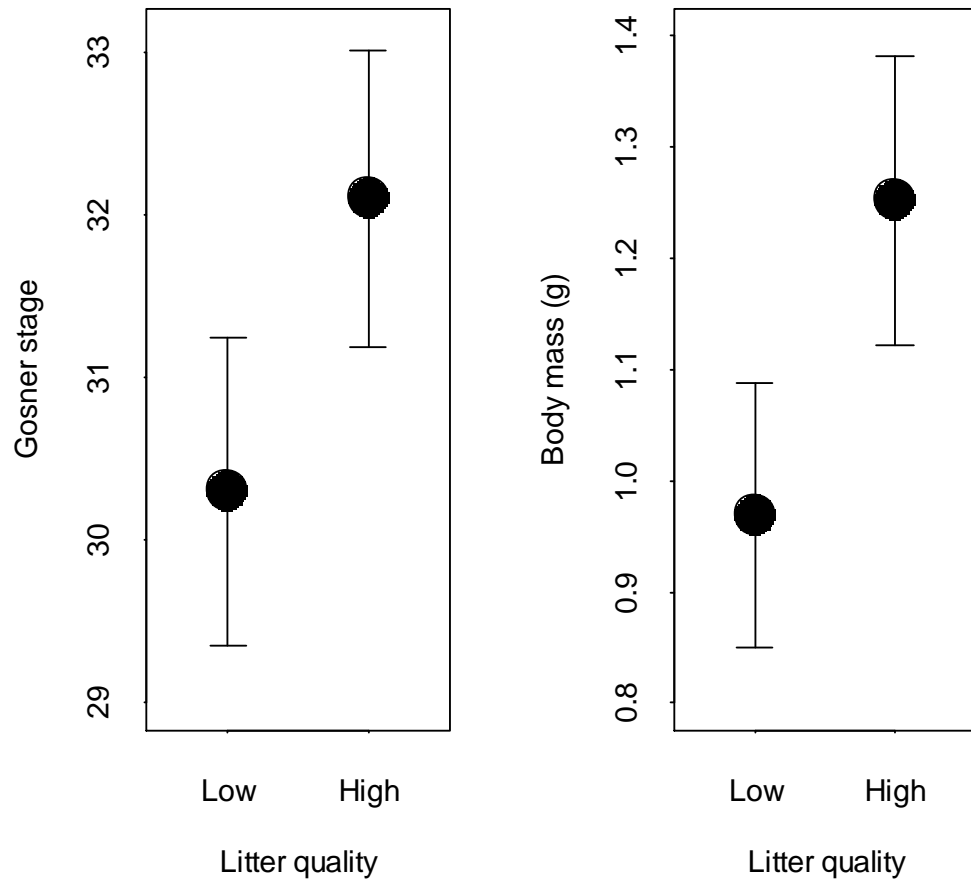


Figure 3.3 *Rana clamitans* development (Gosner stage) and body mass (g) in high or low quality *Phragmites australis americanus* litter. Data are means \pm 2 SE of 10 replicates per treatment.

Discussion

The primary goal of this study was to examine whether changes to microbial communities that accompany plant invasions would affect the performance of *L. clमितans* tadpoles. While we found evidence that soil communities can alter rates of litter mass loss, we found no evidence that these differences in microbial community function trickle up the food web to affect tadpoles. Tadpoles responded only to litter quality, developing faster and growing larger when reared in high quality litter. This finding augments a larger body of evidence that tadpole performance is enhanced with higher quality plant litter (Maerz et al. 2005, Brown et al. 2006, Cohen 2009, Maerz et al. 2010a, Cohen et al. 2012a). We expected that litter mass loss and *L. clमितans* performance would coincide, and would therefore respond uniformly to our experimental treatments, but this was not the case. Tadpoles performed better in litter with lower C:P ratio, and did not respond to soil inoculum the way litter mass loss did. Phosphorus is often a limiting nutrient for freshwater organisms (Schindler 1977, Sterner and Elser 2002), and previous experiments indicate that tadpole performance improves as litter C:P ratio declines (Cohen 2009, Cohen et al. 2012a). Moreover, the two litter populations differed more in C:P ratio than C:N ratio or lignin content, so it is likely that differences in P content caused *L. clमितans* performance to diverge.

Several studies show that plant invasions alter microbial community composition and function (Kourtev et al. 2002, Hawkes et al. 2005, Hawkes et al. 2006, Holly et al. 2009). While we did not directly assess the composition of microbial communities, work on soil pathogens in *Phragmites* patches in the same wetlands (Crocker and Nelson, pers. comm) show that invasive *P. australis* rhizosphere soils are dominated by a distinct set of taxa, supporting our assumption that invasion of *P. australis* alters microbial community composition. Our results

indicate that soils conditioned by introduced *P. australis* harbor microbial communities that accelerate decomposition of high quality plant litter.

The pattern we observed was consistent with our expectations based on parent plant origin. Soil inocula collected under native *P. australis americanus* decomposed lower quality litter the fastest, while soil inocula collected under introduced *P. australis* decomposed higher quality litter the fastest – however this pattern was generally not statistically significant. It is important to note that our source patches for microbial inocula show a narrower range in home litter traits compared to the two litter types we collected in Indiana and Utah (Table 1, Fig. 2). A stronger gradient in source litter quality could potentially result in stronger differences in microbial community function and stronger relationships between ‘home’ litter traits and the ability of microbial communities to decompose litter.

We found no evidence that adding microbial communities from different *P. australis* populations has any effect on tadpoles. Thus, the important function of microbial communities in decomposing litter and providing resources for higher trophic levels was not measurably affected in our experiment. We do not suggest that plant invasions and changes in microbial communities that accompany these invasions may not affect tadpoles. However, our data suggest that if such effects exist, they result from plant-soil feedbacks that ultimately change plant species distributions (Inderjit and van der Putten 2010). If microbial communities play a role in changing plant species distributions, and hence, changing the chemical quality of litter inputs to freshwater systems, then they could have indirect, but important, effects on tadpole performance.

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CHAPTER 4

COMMUNITY-WEIGHTED MEAN FUNCTIONAL EFFECT TRAITS DETERMINE LARVAL AMPHIBIAN RESPONSES TO LITTER MIXTURES

Abstract

Plant species composition is changing across many landscapes, but it is unclear how these changes affect habitat quality for animals. We used functional diversity and community-weighted mean trait values for four plant traits (litter nitrogen [N], phosphorus [P], lignin and soluble phenolics) to explore how changes in plant species composition may affect larval amphibians in a simplified aquatic ecosystem. We predicted that increased functional diversity would improve amphibian performance. We also predicted that increases in community-weighted mean N and P would improve amphibian performance, while increases in community-weighted mean lignin and soluble phenolics would have negative effects on amphibian performance. We did not detect an effect of functional diversity; instead, community-weighted mean litter N and soluble phenolics were useful predictors of amphibian performance. We demonstrate that quantifying the community-weighted mean of ecologically relevant traits represents a powerful approach for predicting how changes in plant species composition can affect aquatic communities.

Introduction

Understanding linkages between biodiversity and ecosystem function is a central endeavor of ecology (Hooper et al. 2005, Hillebrand and Matthiessen 2009). Initially focused on how the number of species in a community affects function, researchers are increasingly interested in linking the distribution of species' traits to function (Petchey and Gaston 2006, Hillebrand and Matthiessen 2009, Cadotte et al. 2011).

Traits influencing ecosystem function, or “functional effect traits” (Violle et al. 2007), and the diversity of these traits, or “functional diversity” appear to be better predictors of ecosystem function than traditional metrics of biodiversity (Cadotte et al. 2011). Improving our understanding of how functional diversity affects ecosystem function will improve predictions of how changes in species composition may affect ecosystems and the services they provide.

Most studies of biodiversity and ecosystem functioning focus on living plants, but dead plants also play significant roles in ecosystems. Plant litter is an important resource for myriad consumers, particularly in freshwater environments (Wetzel 1995) where it can have strong effects on larval amphibians (Maerz et al. 2005, Williams et al. 2008, Maerz et al. 2010b, Earl et al. 2011, Rittenhouse 2011, Stoler and Relyea 2011, Adams and Saenz 2012, Cohen et al. 2012a, Cotten et al. 2012). Litter traits that influence larval amphibian performance include phenolics, lignin, and carbon (C): nitrogen (N): phosphorus (P) ratios (Maerz et al. 2005, Maerz et al. 2010b, Cohen et al. 2012a). Increases in phenolics and lignin tend to decrease tadpole performance (though not for all species, Maerz et al. 2005), while decreases in C:N:P ratios tend to increase tadpole performance (Maerz et al. 2010b, Cohen et al. 2012a).

Though many studies demonstrate that leaf litter influences tadpole performance, the exact mechanisms are unknown. Most tadpoles are opportunistic omnivores - grazing on the mixture of algae, fungi, bacteria and protists that develop on litter surfaces - but diets vary substantially across species and with age (Altig et al. 2007, Schiesari et al. 2009). It is likely that litter traits affect tadpoles in two ways: first, via direct effects of leached materials, such as phenolics, which appear to be toxic to some larval amphibians (Maerz et al. 2005, Brown et al. 2006, Leonard 2008, Watling et al. 2011, Adams and Saenz 2012); and second, via indirect effects on the quality and productivity of biofilms that develop on the litter surface. Here, we build

on evidence from previous studies of single species litter effects on amphibians in trying to develop a predictive understanding of how tadpoles will respond to litter mixtures.

Given observations of how larval amphibians respond to litter from individual plant species, we surmised that litter mixtures could affect amphibians in multiple ways. First, a litter mixture with species ranging in quality from labile (low C:N:P ratios, lignin and phenolics) to recalcitrant (high C:N:P ratios, lignin and phenolics) may make resources available on a more sustained basis than individual litter species would provide, which could be particularly important for slow-developing tadpole species. Second, a litter mixture may offer a more optimal mix of resources compared to single species. For example, some litter species may be high in N but low in P, or vice versa, and both these nutrients can limit larval amphibian growth (Schiesari 2006, Maerz et al. 2010b, Cohen et al. 2012a). Moreover, C-rich litter may provide more carbohydrates, which support tadpole growth, while N-rich litter may provide more protein, which is necessary for development (Richter-Boix et al. 2007). Having a functionally diverse (i.e. different amounts of C, N, and P) litter community may therefore provide a more optimal resource base than any one litter species alone. Third, certain amphibian species are sensitive to phenolics (Maerz et al. 2005); increased litter functional diversity may dilute negative effects of these compounds. Fourth, mixing litter frequently has non-additive effects on decomposition, whereby the amount of mass loss in a litter mixture is different from the amount of mass loss expected from each species decomposing in isolation (Gartner and Cardon 2004). Though the mechanisms underlying this phenomenon remain unclear, non-additive effects tend to be synergistic (i.e. mixtures decompose faster than expected based on decomposition rates of each species in isolation, Gartner and Cardon 2004), with the magnitude of synergistic effects increasing with litter functional diversity (Lecerf et al.

2011). This suggests that a functionally diverse mixture may hasten decomposition, ultimately expediting the transfer of energy and nutrients to tadpoles. Moreover, litter quality could control the magnitude and directions of non-additive effects; high-nutrient litter may enhance the decomposition of other litters (synergy), while litter with inhibitory compounds (i.e. lignin and phenolics) may inhibit decomposition (antagonism, Seastedt 1984).

While there is certainly reason to suspect that litter functional diversity will affect litter decomposition and amphibian performance, an alternative hypothesis is that amphibian performance will simply be a function of the amount of N, P, lignin, and phenolics in the litter - regardless of underlying functional trait diversity. As Grime (1998) postulated, if the traits of a species have an effect on ecosystem functions or processes, then those effects will be proportional to the relative mass of that species in the community (i.e. the “mass ratio hypothesis”). Recent studies show that the “community-weighted mean” (CWM) - obtained by taking the mean trait value for a given species weighted by its relative abundance within the community, then summed across all species (Garnier et al. 2004) - predicts ecosystem function better than functional diversity metrics (Fortunel et al. 2009, Laughlin 2011, Roscher et al. 2012).

In this experiment, we examined relationships between litter mixture traits and the performance of larvae of two common amphibians. We tested predictions that (A) increased litter functional diversity would improve the performance (measured as survivorship, developmental rate, and body size) of each species; (B) mixing litter would have synergistic effects on amphibian performance; (C) amphibian performance would increase as CWM litter nutrient availability (N and P) increased and as CWM lignin and soluble phenolics decreased, and (D) synergistic effects on amphibian

performance would increase with CWM N and P and decrease with CWM lignin and soluble phenolics.

Materials and Methods

In fall 2010 we collected senesced (but not abscised) leaves of introduced *Alnus glutinosa* (black alder), *Typha angustifolia* (narrowleaf cattail), and *Phragmites australis* haplotype M (common reed), and native *Acer rubrum* (red maple) and *Schoenoplectus taebernaemontani* (softstem bulrush). All species are common to larval amphibian habitats in our region. We selected them from a larger pool of species based on previously measured diversity and distribution of traits of interest. We air-dried leaves for three days, prepared samples (125 g each) of all litter types and stored them in paper bags at room temperature. In November 2010, we submitted three samples from each population for N (Cornell Nutrient Analysis Lab in Ithaca, NY, USA) and lignin and P (Dairy One, Ithaca, NY) analyses but used previously obtained phenolic measurements (2007) from the same or nearby collection locations (see Cohen et al. 2012a for details).

In April 2011, we assembled outdoor experimental pond communities (cattle tanks containing 1000 L aged tap water, plant litter, a pond slurry, zooplankton, and tadpoles). Our design consisted of six single litter species treatments and every possible combination of three species, yielding 15 distinct litter treatments. We replicated treatments three times, for 45 mesocosms total. We arranged tanks in five evenly spaced rows of nine on an open, flat area, covered tanks (2 mm screen) and allowed tap water to age for 3 days before adding 375 g of leaf litter. Litter treatments were randomly assigned among tanks. On 02-May-2011, we added 2 L each of filtered pond water (153 μm ; Cornell Experimental Ponds Facility, Ithaca, NY) and a 5 x 5 cm unglazed, ceramic tile (to collect periphyton) to the bottom center of each

tank. On 05-May-2011, we added 40 mL of a well-mixed *Daphnia pulicaria* culture (source: Hairston lab, Cornell University) to each tank. Our intent in introducing a pure culture of *D. pulicaria* was to simulate communities found in natural wetlands while avoiding introducing organisms that may prey on tadpoles (Rittenhouse 2011, Hamilton et al. 2012).

We collected ten *Lithobates sylvaticus* (wood frog) egg masses from a pond in Richford, New York on 22-Apr-2011 and five *Lithobates palustris* (pickerel frog) egg masses at the Arnot Forest in Van Etten, New York on 08-May-2011 (for rearing procedures see Cohen et al 2012). We added 50 *L. sylvaticus* tadpoles ($0.07 \text{ g} \pm 0.002 \text{ SE}$) on 8 May and 25 *L. palustris* tadpoles ($0.02 \text{ g} \pm 0.002 \text{ SE}$) on 23 May to each tank, sampling equally among egg clutches. On 23 May, we recorded water temperature, dissolved oxygen, and pH in each cage using a YSI 556 MPS (YSI Environmental, Yellow Springs, OH, USA). On 10 June we collected tiles and placed them in a cooler for transport to the lab. In a darkened room, we scrubbed and rinsed tiles immediately and filtered ($0.7 \mu\text{m}$) the resulting slurry. We stored filters in a dark dessicator at -20°C until 15 August, when we analyzed them for chlorophyll-a (see Cohen et al. 2012a). Beginning 1 June we surveyed each tank daily and collected metamorphic *L. sylvaticus* (Gosner 1960 stage 41 or higher). We placed individuals in separate containers on moist paper towels until they completed metamorphosis (1-3 days); we then determined their wet weight (0.01 g). We found the first *L. palustris* metamorph on 1 July, and we also observed *L. palustris* tadpoles that appeared to be at very early stages of development (Gosner 30 or below), indicating that they may not reach metamorphosis (tadpoles are known to overwinter, Collins and Lewis 1979, DeGraaf and Rudis 1983). We decided to collect all individuals the following day, which allowed us to assess Gosner stage and size of all individuals on the same day. We collected a 1 L water sample from the middle water column to assess *D. pulex*

abundance, then collected all remaining tadpoles, and all remaining litter from each tank. On 2 and 3 July we assessed Gosner stage and snout-vent length for all remaining *L. palustris* tadpoles (all *L. sylvaticus* tadpoles had either died or metamorphosed), then released them back to their natal pond. We preserved *D. pulex* in 90% ethanol and counted them in August. We dried litter at 60°C for 72 hours and weighed it to the nearest 0.01 g.

We used linear regressions to evaluate the prediction that litter decomposition and amphibian performance would increase as litter functional diversity or community-weighted mean litter traits increased. There are many indices of functional diversity available today (Schleuter et al. 2010). Mason et al. (2005) suggested that to capture functional diversity one needs three complimentary, independent metrics. They suggested functional richness, which measures the volume of multidimensional space created by plotting the trait values for all members of the community; functional evenness, which measures the regularity of distribution of abundance within that volume; and functional divergence, which measures how species diverge from the center of gravity within that volume (for a detailed explanation see Mason et al. 2005). Of the metrics Mason et al. (2005) propose, only functional richness can characterize the ‘range’ of trait values. However, Villéger et al. (2008) pointed out several limitations of functional richness; namely, that it cannot incorporate information on abundances, is sensitive to outliers, and cannot be used when the number of traits is greater than the number of species in the community (as was the case in our experiment). They proposed functional dispersion as an alternative metric. Functional dispersion calculates the mean distance of individual species’ trait values from the centroid of all species trait values (Villéger et al. 2008). We therefore chose to use functional dispersion, functional evenness, and functional divergence to measure functional diversity.

To understand the effects of functional diversity on our experimental ecosystem we calculated functional dispersion, functional evenness and functional divergence using the FD package (Laliberte and Legendre 2010) in R (R Development Core Team 2011). We used linear regressions with each functional diversity metric as the independent variable. For each model, dependent variables included chlorophyll-a content, *D. pulex* abundance, amphibian performance metrics (number reaching metamorphosis, days to metamorphosis, and mass at metamorphosis for *L. sylvaticus*; number, Gosner stage, and snout-vent length for *L. palustris*), or litter mass loss. We repeated this protocol using CWM litter N, P, lignin and soluble phenolics as independent variables. Finally, to examine litter-mixing effects, we calculated signed deviation (observed value - expected value, Lecerf et al. 2011) for litter mass remaining and each amphibian performance metric. We performed linear regressions with signed deviation as the dependent variable and functional diversity metrics or CWM litter traits as independent variables.

Results

Conditions in tanks with only *A. rubrum* litter proved deadly to both *L. sylvaticus* and *L. palustris* tadpoles, causing nearly 100% mortality. Measurements indicated significantly lower dissolved oxygen and lower pH in these treatments and we excluded this treatment from analyses of amphibian performance as it would severely undermine assumptions of normality and heteroscedasticity. Tadpole survival in all other treatments was high (77% and 96% for *L. sylvaticus* and *L. palustris*, respectively).

Contrary to our prediction, we did not detect any significant relationship between any metric of amphibian performance and any functional diversity metric (Figure 4.1). Instead, we found significant relationships between amphibian

performance and CWM N and phenolics in leaf litter (Table 4.1, Figure 4.2). The number of *L. sylvaticus* reaching metamorphosis increased as CWM litter N increased (Figure 4.2A) and time to metamorphosis increased as CWM litter phenolics increased (Figure 4.2B); none of the litter traits had a significant relationship with *L. sylvaticus* mass at metamorphosis. We did not detect a relationship between *L. palustris* survival and CWM N, P, lignin or phenolics. However, *L. palustris* developmental rates and snout-vent length increased as CWM litter N increased (Figure 4.2C, D); in addition, developmental rates increased as CWM litter phenolics decreased (Table 4.1).

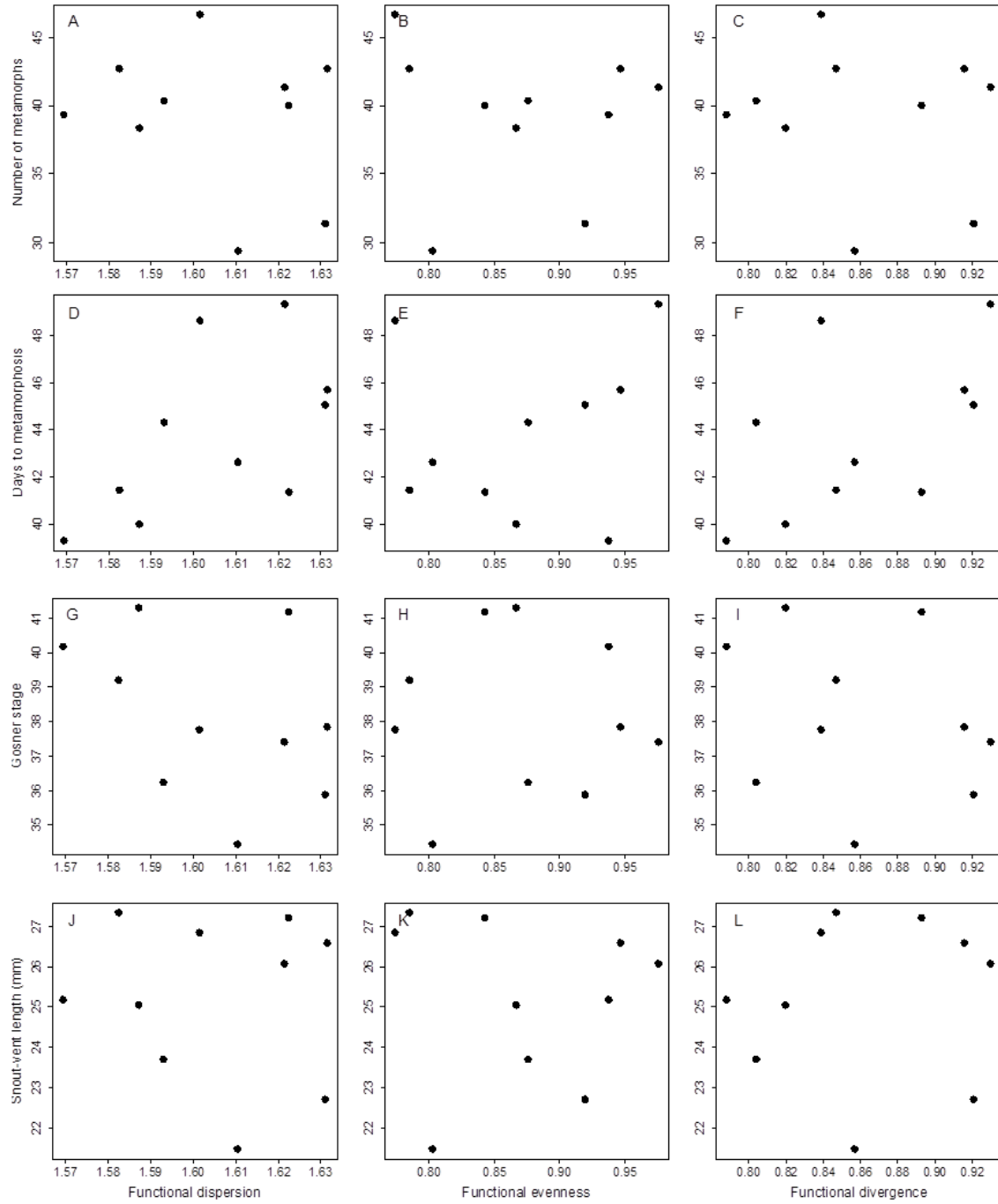
Litter mixture effects also responded to CWM litter N and phenolics (Figure 4.3). The effect of litter mixture on the number of *L. sylvaticus* achieving metamorphosis became more antagonistic (signed deviation decreased, fewer tadpoles achieved metamorphosis) as CWM phenolics increased (Figure 4.3A). For *L. palustris* developmental stage and snout-vent length, mixture effects became more synergistic (signed deviation increased, more tadpoles achieved metamorphosis) as CWM N increased (Figure 4.3B, C).

Litter mass loss was variable among treatments but best explained by a model that contained CWM litter N, P, and soluble phenolics (Table 4.1); there was no relationship between litter mass loss and either functional dispersion, functional evenness, or functional divergence. Chlorophyll-a and *D. pulex* abundance were not related to functional diversity or to CWM N, P, lignin, or phenolics.

Table 4.1 Models of amphibian performance and litter mass loss as a function of community-weighted means (CWM) for four litter traits. (*) indicates that $P < 0.05$, (**) indicates that $P < 0.01$, (***) indicates that $P < 0.001$.

Response variable	Independent variables (CWM)				Model adjusted r^2
	N	P	Lignin	Soluble phenolics	
Number <i>Lithobates sylvaticus</i> reaching metamorphosis	4.08	-2.29	-0.87	0.12	0.20
<i>L. sylvaticus</i> days to metamorphosis (d)	-1.43	1.25	0.34	0.77***	0.82***
<i>L. sylvaticus</i> mass at metamorphosis (g)	0.11	0.83	-0.02	-0.01	0.12
Number of <i>L. palustris</i> tadpoles	1.02	-5.70	-0.02	-0.08	-0.10
<i>L. palustris</i> Gosner stage	3.52*	-13.01	-0.30	-0.25	0.64**
<i>L. palustris</i> snout-vent length (mm)	3.63**	-4.73	-0.03	-0.08	0.64**
Litter mass remaining (g)	-29.82*	668.58*	5.51	-7.01**	0.80***
Chlorophyll - a	4.23	15.31	1.16	-0.21	-0.008
Number of <i>Daphnia pulex</i>	-31.25	-991.20	31.36	6.14	-0.04

Figure 4.1 Performance of *Lithobates sylvaticus* (number of metamorphs, A, B, C; and days to metamorphosis, D, E, F) and *L. palustris* (Gosner stage, G, H, I; and snout-vent length, J, K, L) as a function of functional dispersion, functional evenness, and functional divergence. Circles represent mean values ($n = 3$) for each litter community.



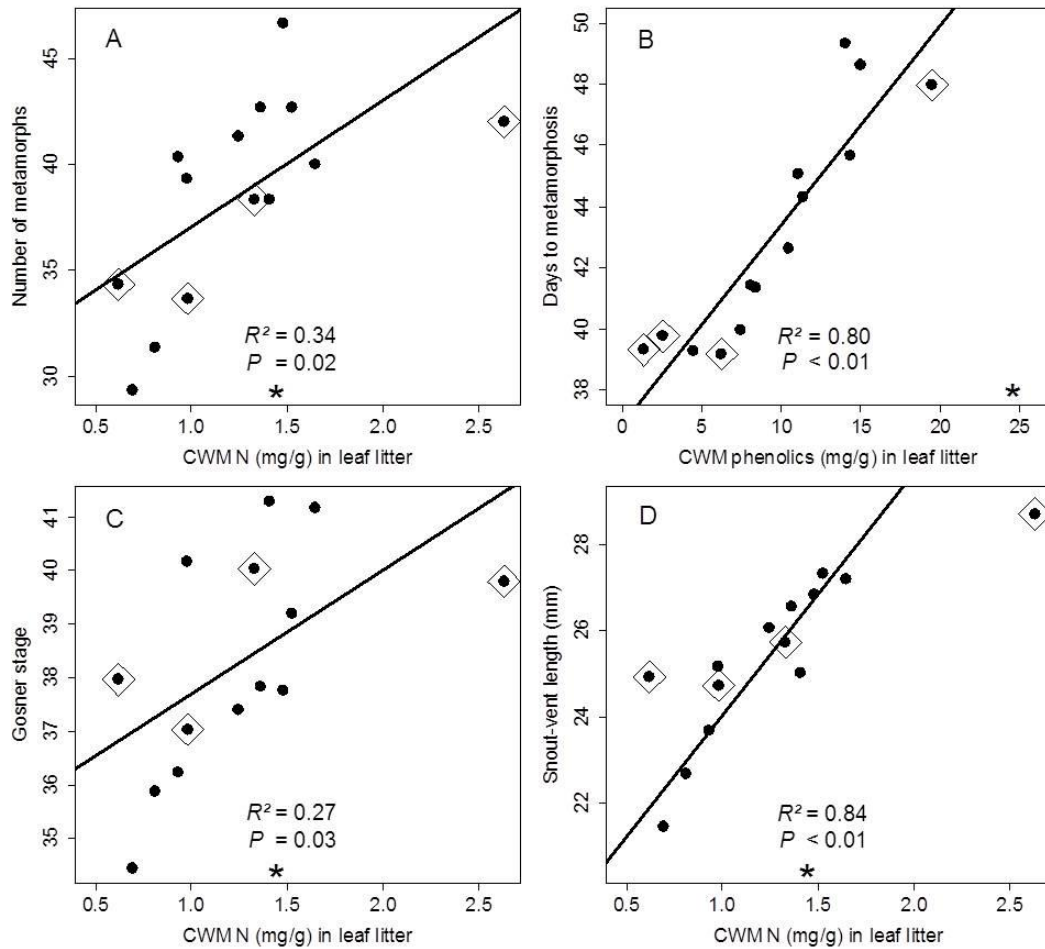
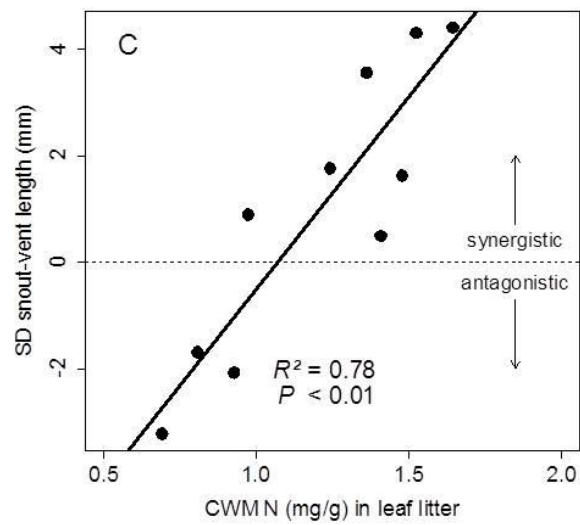
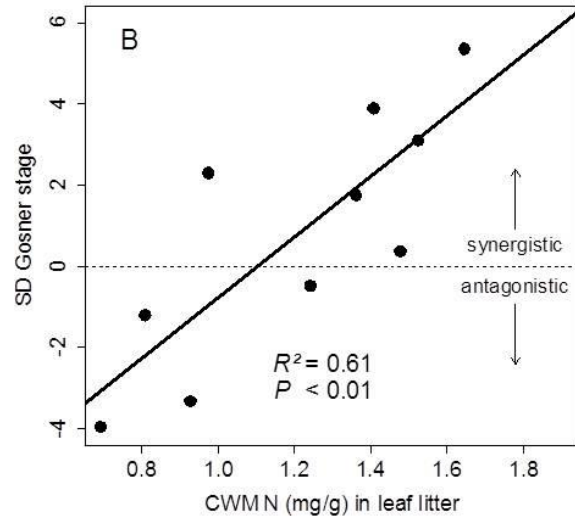
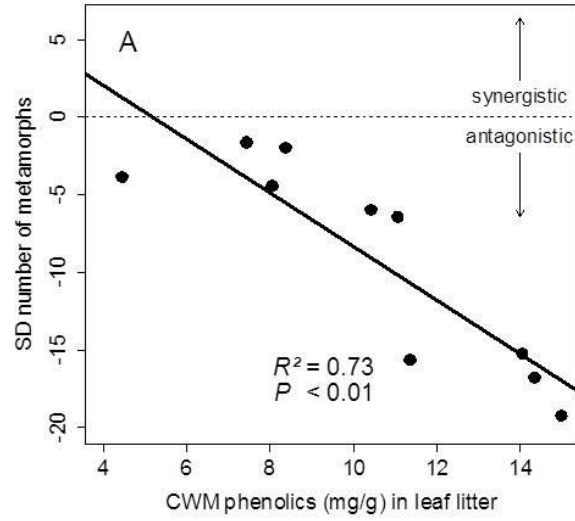


Figure 4.2 Number of *Lithobates sylvaticus* reaching metamorphosis (A), *L. sylvaticus* days to metamorphosis (B), *L. palustris* developmental stage (Gosner stage, C), and *L. palustris* snout-vent length (D) as a function of CWM litter N or CWM litter phenolics. Circles represent mean values ($n = 3$) for each litter community, squares denote single-species treatments. An asterisk (*) indicates CWM litter N or CWM litter phenolics for *A. rubrum*-only mesocosms as a reference; this was not incorporated into the analysis. Solid lines represent linear regressions with each performance metric as a function of CWM litter N or CWM litter phenolics.

Figure 4.3 Days to *Lithobates sylvaticus* metamorphosis (A) and developmental stage of *L. palustris* (Gosner stage, B) as a function of community-weighted mean soluble phenolics found in litter treatments. Circles represent the mean value ($n = 3$) for each litter community, squares denote single-species treatment. An asterisk (*) indicates community-weighted mean phenolics in *A. rubrum* only mesocosms as a reference; this value was not incorporated into the analysis. Solid line represents linear regression with each performance metric as a function of CWM litter phenolics.



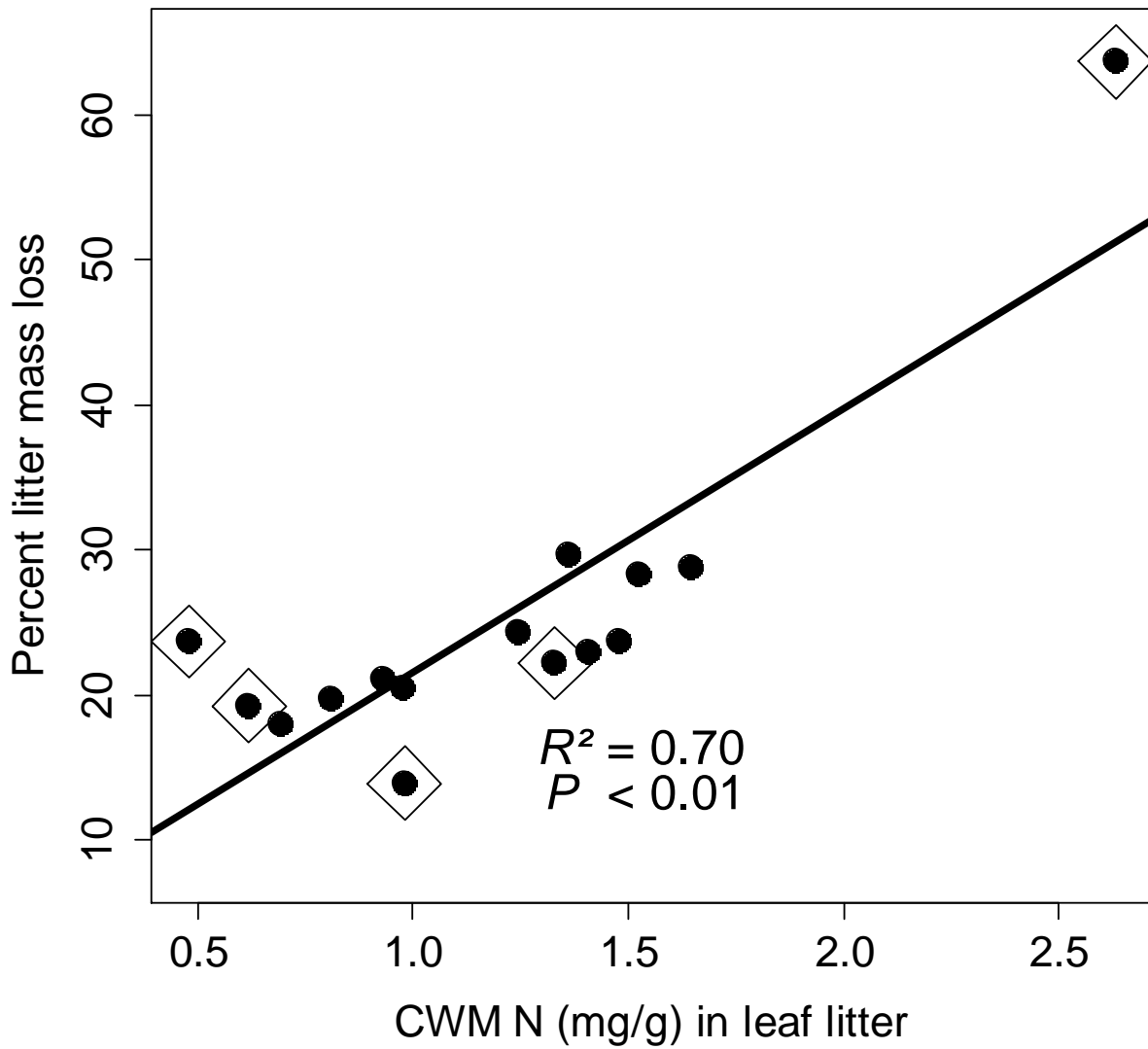


Figure 4.4 Percentage of litter mass lost as a function of CWM litter N. Circles represent mean values ($n = 3$) for each litter community, squares denote single-species treatments. Solid line represents a linear regression with percentage litter mass loss as a function of CWM litter N.

Discussion

Contrary to our expectations, litter functional diversity had no measurable effect on litter decomposition or amphibian performance in our experiment. Instead, we found (A) strong species-specific effects of *A. rubrum*; (B) that amphibian performance was best predicted by CWM litter traits, and (C) that CWM litter traits determined the direction and magnitude of litter-mixing effects on amphibians. Increases in CWM litter N had positive effects on both amphibian species, while increases in CWM phenolics had negative effects.

Many studies have found that functional diversity does not influence litter decomposition (Wardle et al. 1997, Hoorens et al. 2003, Schindler and Gessner 2009, Barantal et al. 2011). However, these studies typically use only one metric of functional diversity. Functional diversity is complex, and single metrics may not capture its effects (Mason et al. 2005). For example, Lecerf et al. (2011) detected a significant effect of functional redundancy on litter decomposition, but found no effect of functional richness or divergence, suggesting that it is important to examine all facets of functional diversity in order to detect an effect on decomposition. In this study, we used three metrics of functional diversity, yet we still did not find evidence for a relationship between litter functional diversity and decomposition, or for litter functional diversity and any other response parameter.

Wardle (1997) proposed that functional diversity is important when niche complementarity drives ecosystem function, as when living plants partition resources with the result of increased biomass production. Based on this theory, we hypothesized that litter functional diversity would enhance amphibian survivorship and performance by provisioning resources at different times. We reasoned that labile species would provide an initial pulse of resources, while recalcitrant species would provide resources later on; in this way, more functionally diverse mixtures could

provide a more reliable, sustained resource base that would improve overall amphibian performance. Our results did not support this hypothesis. Litter species decomposition was very different (e.g. *A. glutinosa* lost more than 80% of its mass, while *S. taebernaemontani* lost less than 30%) and our amphibians had different phenologies - all *L. sylvaticus* completed metamorphosis before the first *L. palustris* began it - yet litter functional diversity did not affect survivorship or performance of either amphibian.

It is possible that we did not see a relationship between functional diversity and amphibian performance because our diversity metrics contained traits that were not relevant for amphibian performance. Based on evidence from a previous experiment (Cohen et al. 2012a), we suspected that litter P and lignin would influence amphibian performance. However, we did not detect effects of litter P or lignin in this experiment. For example, amphibians performed well in tanks with *T. angustifolia* only, even though these tanks had 35% less P and 73% more lignin than the average across all treatments. To test the idea that we used “ineffective” functional effect traits, we re-calculated functional dispersion, functional evenness and functional divergence excluding litter P and lignin, but we still failed to find significant relationships for litter mass loss or for any of our amphibian response metrics (data not shown).

Within our experiment, we find a realization of all possible effects on consumers - positive, neutral, and antagonistic (Bishop and Kelaher 2008, Kominoski and Pringle 2009, Sanpera-Calbet et al. 2009, Stoler and Relyea 2011). However, our focus on ecologically relevant traits revealed a distinct pattern that may explain the magnitude and direction of litter mixing effects. As CWM N increased, effects of litter mixtures on amphibians became increasingly synergistic – mixing high N species resulted in amphibian performance that was better than one would predict based on the average of amphibian performance for those species in isolation. The opposite was

true for phenolics; as CWM phenolics increased, litter mixture effects became increasingly antagonistic. This suggests that increasing plant species richness is only ‘good’ for amphibians if it increases the value of ‘good’ traits in the plant community. Additional evidence for this suggestion arises when one looks specifically at *A. rubrum*. We observed that *A. rubrum* was highly lethal to both amphibian species, yet animals reared in tanks with *A. rubrum* litter fared much better when *A. rubrum* was mixed with other species. *Acer rubrum*-only tanks had two to three times more soluble phenolics compared to other treatments. In *A. rubrum*-only tanks dissolved oxygen levels dipped below 2 mg L^{-1} , such that there was not enough oxygen for tadpoles to survive (Leonard 2008, Adams and Saenz 2012). When *A. rubrum* litter was mixed with other species, oxygen levels were similar to treatments without *A. rubrum*, suggesting that litter mixtures can dilute negative impacts from harmful species.

Generations of ecologists have investigated how the distribution of plant species affects the distribution of animals, and the question remains relevant to this day – perhaps more so, given how quickly plant species composition is shifting across many landscapes. In this study, we used a trait-based approach to assess how a simplified ecosystem would respond to ten different combinations of plant species. We found that CWM traits explained much of the variation in the response metrics we measured. While we carried out our experiment in mesocosms that mimic freshwater wetlands of the Northeastern United States, the trait-based approach used here represents a powerful tool for predicting how changes in plant species composition may affect animals in any ecosystem.

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